ijlpr 2023; doi 10.22376/ijlpr.2023.13.5.P241-P254

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Chemo metric Assisted RP-HPLC for Simultaneous Estimation

Development and Validation of Chemometric Assisted RP-HPLC Method for Simultaneous Estimation of Perindopril Erbumine, Indapamide, and Amlodipine Besylate in Bulk and Pharmaceutical Formulation

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Abstract: This research paper illustrates a latterly developed, optimized and validated gradient RP-HPLC approach for simultaneous analysis of Indapamide, Perindopril erbumine and Amlodipine besylate in bulk and pharmaceutical formulation with the assistance of quality by design. Quality is predicated on desired and predetermined specifications. Understanding various factors, dependent variables, and their interconnection effects by a desired set of experiments on the responses to be analyzed is an important component of QbD. Several operating conditions of various processes optimization, chromatographic separation performance improvement, and high extraction efficiency were attained by using QbD. The powerful chromatographic conditions were done using the HypersilC_{18} column (250mm × 4.6mm, 5µm particle Size). The UV detector was adjusted to 215nm. Design of experiments (DoE) was applied for multivariate optimization of the experimental conditions of the RP-HPLC method. Three independent factors, mobile phase composition, phosphate buffer strength, and flow rate, were used to design mathematical models. Central composite design (CCD) was used to examine the response surface methodology and fully examine the results of these independent factors. The desirability function was used to optimize the retention time and resolution of the analytes simultaneously. The improved and anticipated data from the contour diagram consisted of methanol and phosphate buffer (pH 2.5, strength 0.05M) in the ratio of 65:35, respectively, at a flow rate of 1.1 ml/min. Using these optimum conditions, baseline separation of both drugs with good resolution and run time of less than 5.0 min was achieved. The novelty of the developed method was time-consuming, cost-effective, and sensitive. The optimized assay conditions were validated according to ICH guidelines. Under the optimized state, the linearity ranges were found to be 10-40 µg/mL, 32–128 µg/mL, and 40-160 µg/mL for Indapamide, Perindopril erbumine, and amlodipine besylate, respectively, with correlation coefficients (R^2) of 0.999. The mean accuracy studied ranged from 99.18 to 99.58%. The percentage coefficient variation value for the precision study was lower than 1%. The proposed method showed good precision and repeatability. Hence the developed RP-HPLC method using quality by design can be used as a routine quality control analysis of indapamide, perindopril erbumine, and amlodipine besylate.

Keywords: Central Composite Design, Optimum conditions, Ruggedness Robustness, Method development, Validation

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Received On 18 November, 2022

Revised On 5 June, 2023

Accepted On 26 June, 2023

Published On 1 November, 2023

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Thomas Sudha, Palani Kumar Nallasivan, Munnusamy Vijayakumar Saranya and Arumugam Sarveswaran , Development and Validation of Chemometric Assisted RP-HPLC Method for Simultaneous Estimation of Perindopril Erbumine, Indapamide, and Amlodipine Besylate in Bulk and Pharmaceutical Formulation.(2023).Int. J. Life Sci. Pharma Res.13(5), P241-P254 http://dx.doi.org/10.22376/ijlpr.2023.13.5.P241-P254

1. INTRODUCTION

Perindopril Erbumine (Fig.1)[1] is chemically described as a (2S,3∝S,7∝S)1 [(S) N [(S) 1 Carboxybutyl]alanyl] hexahydro 2 indolinecarboxylic acid, 1ethyl ester, compound with tertbutylamine (1:1). It is the butylamine salt of perindopril, the ethyl ester of a non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor with antihypertensive activity. Upon hydrolysis, perindopril erbumine is converted to its active form, perindoprilat, inhibiting ACE and the conversion of angiotensin 1 to angiotensin II; consequently, angiotensin II-mediated vasoconstriction and angiotensin II stimulated aldosterone secretion from the adrenal cortex are inhibited and diuresis and natriuresis ensue.Indapamide (Fig. 2)[2] is chemically described as a 4-chloro N(2 methyl 2,3 dihydro1H indol 1 yl)3 sulfamoylbenzamide. Thiazide-like diuretics (indapamide and chlorthalidone) appear more effective than thiazide-type diuretics (hydrochlorothiazide) in reducing the risk of major cardiovascular events and heart failure in persons with high blood pressure. Amlodipine besylate (Fig. 3) 3 known as 3 Ethyl 5 methyl (±)2[(2 aminoethoxy) methyl] 4 (2 chlorophenyl)1,4 dihydro methyl 3,5 pyridinedicarboxylate. It is the besylate salt of amlodipine, a synthetic dihydropyridine with antihypertensive and antianginal effects. Amlodipine inhibits the influx of extracellular calcium ions into myocardial and peripheral vascular smooth muscle cells, thereby preventing vascular and myocardial contraction. This dilates the main coronary and systemic arteries, decreases myocardial contractility, increases blood flow and oxygen delivery to the myocardial tissue, and decreases total peripheral resistance[4-6]. The literature survey reported UV[7], HPLC[8], and HPTLC[9] methods for determining three analytes. Determination of these APIs alone or in dual combination methods has been reported[10-19]. But the literature survey revealed no published method for the simultaneous RP-HPLC estimation of perindopril erbumine, indapamide, and amlodipine besylate bulk and in pharmaceutical dosage forms using Derringer's desirability function. The pharmaceutical company develops a new strategy to add or remove contemporary quality and risk management systems required for product safety, efficiency, efficacy, and safety[20]. In all regulatory bodies, quality is the principal standard have more importance for any entity. A new drug product development consists of several pharmaceutical procedures and analytical testing. These analytical test reports reinforce further determine how development should be followed [21]. These days frequently, analytical method development is failure more during method transition. Even though analytical specifications, interferences might happen from analyst, analyte, lab environment, and instrument [22]. To ensure that the system performs well over the product's lifetime, robustness and ruggedness should be developed before the system forming procedure[23].If not introduced prior sufficiently, it could be applicable to revalidate, retransfer and redevelop specifications methodological procedures, which would take more time and spend more money [24]. The product and process ability attribute must be technically engineered to achieve particular targets ensured by using QbD for certain nations. The analytical QbD activities should be carried out before an analytical method development before initiating validation [25]. In chromatographic methods, experiments (DoE) design is an important tool. It not only supports recognition of method variables that have an important effect on method ability, but it also constructs it simple to refine method variables to effort, resources, and save time. The greater success of the QBD methodology in chromatographic method development with greater Several literature studies exists in this respect, demonstrating the greater success of the QbD methodology for the efficient development of chromatographic methods with greater creativity and improved process efficiency[26-30]. It is a trial arrangement that grants analyzing several factors simultaneously in a predetermined number of trials. Experimental designs can be classified into screening designs (e.g., fractional, full factorial, Plackett Burman, response surface, and mixture). HPLC method optimization is a complicated procedure that is an essential simultaneous estimation of many factors (e.g., stationary phase, type, and composition of the organic phase, flow rate, pH, and column temperature) by applying the experimental design method. This current work aims to develop and validate a narrative RP-HPLC method for simultaneously determining indapamide, perindopril erbumine, and Amlodipine besylate by applying a central composite design. The importance of the analyzed factors and optimum chromatographic conditions were determined using a central composite design (CCD) and mathematical global optimization approach (Derringer's desirability function). Finally, the proposed method was tested for linearity, specificity, precision, accuracy, robustness, and ruggedness.

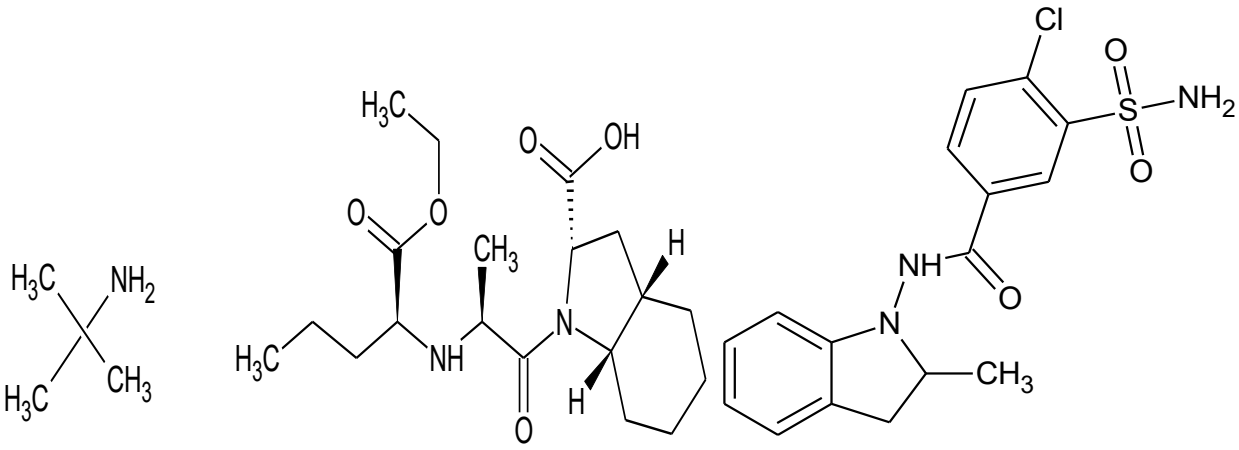

Fig. 1: Perindopril Erbumine Fig. 2: Indapamide

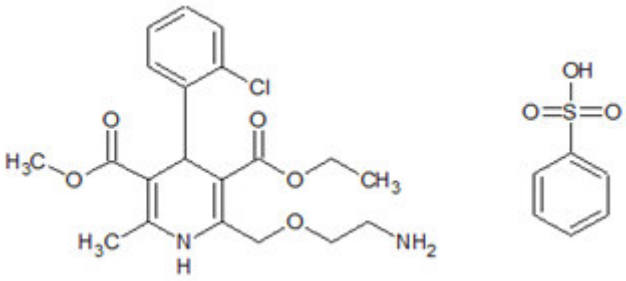

Fig. 3: Amlodipine besylate

2. EXPERIMENTAL

2.1. Reagents and Chemicals

The analytes (Raw materials) Perindopril erbumine, Indapamide, and Amlodipine were gifted from Nebulae Hi-tech Laboratories, Chennai. The pharmaceutical dosage formulation TRIPLIXAM (Serdia Pharmaceuticals Private, limited. Mumbai, Maharashtra, India) containing 4 mg of perindopril erbumine, 1.25 mg of Indapamide, and Amlodipine besylate I.P (equivalent to amlodipine 5mg). The formulation was procured from 1mg (online shopping). All the reagents were prepared by using double distilled water. Acetonitrile (HPLC grade), methanol (HPLC grade), Potassium dihydrogen phosphate (AR grade), and orthophosphoric acid (AR Grade) were purchased from Loba Chemicals (Mumbai, India). Calibrated glassware was used throughout the work.

2.2. Instrumentation

The proposed RP-HPLC method was performed with the Shimadzu HPLC–2030 Plus Prominence-I Series**,** have an elution mode four-solvent low-pressure gradient flow rate range from 0.0001 to 10 mL/min. Degassing unit is five Lines: Mobile phase 4 + Rinse solution 1 (Volume capacity 400 µL). It is an autosampler with a needle in flow path mechanism injection volume range of 0.1-100 µL; oven capacity is six columns at 10 cm maximum, 3 pieces at 10 cm to 30 cm, and UV detector containing wavelength range from 190 to 700 nm. Shimadzu LC-solution software version 6.42 is used for the analysis. Elico LI 120 pH meter was used for the pH measurement of the solution. The prepared solution was sonicated by using the Sonicator model 2120 MH. The chromatographic segregation practiced by C_{18} column Hypersil (250mm × 4.6mm, 5µm particle size) was used as a stationary phase at a 1.0 mL flow rate. The injection volume was 20µL. The segregation was carried out at 50°C, and the UV detector was adjusted to 215 nm. The mobile phase contains a methanol and phosphate buffer (65:35%v/v) with pH adjusted to 2.55 using orthophosphoric acid. Then it was filtered through a 0.45µm membrane filter using a vacuum pump and degassed

2.3. Preparation of Standard Stock Solution

About 25 mg of perindopril erbumine, 25 mg of indapamide, and 25 mg of Amlodipine besylate were weighed accurately and transferred into 50 mL, 100 mL, and 25 mL flasks separately. Dissolved and made up with methanol (HPLC grade). Further dilution was made by pipetting 1.0 mL of each mother liquor and transferring it into the same 10 ml volumetric flask. Then made up the volume with the mobile phase. The concentration of the solution was observed to obtain 80 µg/mL, 25 µg/mL, and 100µg/mL, respectively [31].

2.4. Quantification of Formulation (Assay)

Perindopril erbumine, Indapamide, and Amlodipine Besylate were estimated in tablet formulation by RP-HPLC using optimized chromatographic conditions. Twenty tablets of formulations (TRIPLIXAM) were weighed, and the average weight of the tablet was found and powdered. The tablet powder equivalent to 25 mg of Perindopril Erbumine, Indapamide, and Amlodipine besylate was weighed and transferred into a 25 mL volumetric flask. About 15 mL of methanol was added to dissolve the substance. Then the solution was sonicated for 15 mins. The volume was made up to the required volume with the same solvent and centrifuge at 3000 rpm. Then the solution was filtered through Whatman filter paper No: 41 to get 500µg/mL of Perindopril erbumine, 250 µg/mL of Indapmide, and 1000 µg/mL of Amlodipine besylate. From the clear solution, a further 1.0 mL of this solution was diluted to 10 mL with mobile phase to obtain 80 µg/mL of Perindopril Erbumine, 25 µg/mL of Indapamide, and 100 µg/mL of Amlodipine Besylate theoretically. A steady baseline was recorded with optimized chromatographic conditions. After the baseline stabilization for 30 minutes, the test solutions were injected, and the chromatogram was recorded. The concentration of each test solution was determined by using slope and intercept values from calibration graph [32].

2.5. Experimental design

2.5.1. Assessment Step

Chromatographic trials are evaluated in this step to identify which Mobile phase gives an acceptable (system suitability parameter within the limit) partition between the three analytes. For the first trial, different mobile phases containing either water or potassium hydrogen phosphate buffer as the aqueous part of the mobile phase was tried. In addition, acetonitrile and methanol were tested.

2.5.2. Optimization Study

The Optimization Process Central Composite Design (CCD) was broadly used because of its high effectiveness and ability to decrease run numbers. A CCD with k factors is necessary for 2k factorial runs, 2k axial investigation, symmetrically spaced at ± α further every variable axis, and at least one center point [33]**.** A rotatable CCD (α = 1.68) was built for the three significant factors to apply the optimum level for the proper responses utilizing five levels of each factor (−α, −1, 0, +1, +α) with a total number of 30 random runs including 6

center points. The numerical optimization process and desirability function procedure are generally applied cooperatively for locating the optimized positions by various substitutes of the selected responses [34].

2.6. Method Validation

The International Conference on Harmonization (ICH) requirements[35] validated the method involving system suitability, linearity, LOD, LOQ, accuracy, precision, and robustness.

2.6.1. System suitability studies

The system suitability studies conceded as per ICH guidelines and USP. The parameters like capacity Factor, tailing factor, asymmetry factor, and several theoretical plates were calculated.

2.6.2. Preparation of Calibration solution

The aliquots of stock solution of perindopril erbumine (4-16 mL of 80 µg/mL), indapamide (4-16 mL of 25 µg/mL), and Amlodipine besylate (4-16 mL of 100 µg/mL) individually were transferred into six 10 mL volumetric flasks and made up to mark with the mobile phase. The solutions contained the concentration of 32-128 µg/ml of Perindopril Erbumine, 10-40 µg/mL indapamide, and 40-160 µg/mL of Amlodipine besylate. From this solution, 20 µL was injected, and the chromatogram was recorded at 215 nm. The above concentration range was linear and obeyed Beer's law[36].

2.6.3. Limit of Detection (LOD)and Limit of Quantification(LOQ)

The linearity study was carried out three times. The LOD and LOQ were calculated based on the calibration curve method. The LOD and LOQ were calculated using an average of slope and intercept. The following formula was used to calculate LOD and LOQ values.

$LOD = 3.3 * \alpha/S$ and $LOQ = 10 * \alpha/S$

where α- Standard deviation of intercepts and S- Slope of the calibration curve

2.6.4. Recovery studies

A recovery study determined the accuracy of the method. A recovery study was performed by the standard addition method. The recovery experiment added known concentrations of Perindopril Erbumine, Indapamide, and Amlodipine Besylate working standard to the pre-analyzed formulations. The 80% pre-analyzed formulations solutions, known quantities of standard drug that is 80%,100%, and 120% of quantification concentration (20 µg/mL, 64µg/mL, and 80 µg/mL) were added into a series of 25 ml volumetric flasks, diluted with methanol and sonicated for 15 minutes. After sonication, the solution was made up to 50 mL with methanol. The solution was filtered through Whatman filter paper No.41; from each solution, 1.0 mL of clear filtrate was transferred into a series of 10 ml of volumetric flask and made up to the volume with mobile phase [37].

2.6.5. Precision

The method's repeatability was checked by replacing the formulation analysis six times with the same concentrations. The amount of drug present in the formulations was calculated. The percentage RSD value was calculated[38].

2.6.6. Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (± 0.1 mL/min), the composition of mobile phase (± 3%), and wavelength (± 2nm). For each condition, 20 µL solutions were injected into the chromatographic system, and chromatograms were recorded. The system suitability parameters were checked [39].

2.6.7. 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[40].

3. STATISTICAL ANALYSIS

A Central composite design is suitable for exploring quadratic response surfaces and establishing second-order polynomial models with Design Expert 12 ® (version 7.1.6., trial version). The statistical calculations like Average, Standard deviation, and percentage relative standard deviation (%RSD) were calculated using a Microsoft Excel worksheet.

4. RESULTS AND DISCUSSION

The separation of analytes by applying gradient mode mobile phase containing Acetonitrile: Methanol: H_2O (20:20:60%v/v/v) was tried. This trail reports long tailing, and peaks are merged between the last two analytes. To solve this problem, methanol concentration was increased, the tailing effect was normal and got better resolution, and system suitability parameters were well within the limit. In the mobile phase, instead of a water phosphate buffer, pH 2.6 was used. In this condition, good separation with sharp peaks was obtained. This study selected factors were; factor A: organic solvent concentration, factor B: buffer pH, and factor C: flow rate.In the present study, simultaneous optimization of resolution and retention time, the chemometric protocol of response surface design, and Derringer's desirability functions were profitably working. The central composite design could optimize the partition and help develop a finer perception of the reciprocal action of several chromatographic factors in partition quality. A central composite design experiment chose and optimized the main chromatographic factors in the current study. Factors chosen and optimized were constructed from prior experiments and preliminary skills from the publications. Failure mode and effects analysis (FMEA) are widely used risk assessment tools. The FMEA method is often used to perform a quantitative risk assessment. FMEA is used during the design stage to avoid future failures. Later it is used for process control before and during the ongoing operation of the process. Ideally, FMEA begins during the earliest conceptual stages of design. The outcome of an FMEA development is actions to prevent or reduce the severity or likelihood of failures, starting with the highest-priority ones [41-44]. The factors chosen for the optimization procedure were organic solvent

concentration (A- methanol), buffer pH (B), and flow rate (C). The ranges of factors used were organic solvent (methanol) concentration (65 – 75%), buffer pH (2.4 – 2.8), and flow rate (0.8 – 1.2 mL/min). Three responses were chosen: The capacity factor for the first eluted peak of Indapamide (k_1), the resolution of Perindopril erbumine and Amlodipine besylate peak ($Rs_{2,3}$) and the retention time of the last peak AML (Rt_3) presented in Table -1

Table 1: Experimental Design and Results of a Central Composite Design

Run	Space Type	Factor A MeOH Conc (%v/v)	Factor B PB pH	Factor C Flow Rate (ml/min)	Response 1 k_1	Response 2 $Rs_{2,3}$	Response 3 Rt_3
1	Center	70	2.6	1	1.48	2.59	2.842
2	Factorial	75	2.4	0.8	1.56	2.66	3.51
3	Center	70	2.6	1	1.48	2.59	2.842
4	Center	70	2.6	1	1.48	2.59	2.842
5	Factorial	65	2.4	0.8	1.56	2.68	3.31
6	Axial	70	2.6	1.33636	1.5	2.43	2.27
7	Axial	70	2.93636	1	1.48	2.69	3.042
8	Axial	70	2.26364	1	1.48	2.59	2.642
9	Factorial	65	2.8	1.2	1.5	2.49	2.47
10	Factorial	65	2.4	1.2	1.5	2.48	2.37
11	Center	70	2.6	1	1.48	2.59	2.842
12	Factorial	75	2.8	0.8	1.56	2.67	3.61
13	Axial	61.591	2.6	1	1.41	1.61	2.419
14	Center	70	2.6	1	1.48	2.59	2.842
15	Factorial	65	2.8	0.8	1.56	2.67	3.62
16	Factorial	75	2.4	1.2	1.5	2.46	2.37
17	Axial	78.409	2.6	1	1.44	1.87	2.453
18	Axial	70	2.6	0.663641	1.56	2.65	3.51
19	Factorial	75	2.8	1.2	1.5	2.48	2.47
20	Center	70	2.6	1	1.48	2.59	2.842

PB- Phosphate buffer, k_1- Capacity factor, $Rs_{2,3}$ - Resolution between peak 2 and 3, Rt_3-Rentition time for third peak

All experiments were conducted in arbitrary sequence to reduce the effects of unlimited variables that might initiate a measurement bias. Replicates (n = 6) of the central points were performed to determine the investigational error. For an experimental de$_s$ign with three factors, the model, including linear, quadratic, and cross terms, can be expressed as

$$Y = \beta_0 + \beta 1X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta 11X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$

Y is the response to be modeled, β is the regression coefficients, and X_1, X2, AndX_3 represent factors A, B, and C, respectively. Statistical parameters obtained from ANOVA for the reduced models are given in Table -2.

Table 2: Standard for the optimization of the particular responses for the examination of quality control samples

Response	Regression model	Adjusted R^2	Model *P*-value	(%) C.V	Adequate precision
k_1	+1.48+0.0037*A+0.0000*B-0.0250*C+0.0000*AB+0.0000*AC+0.0000*BC-0.0085*A^2+0.0109*B^2+0.0286*C^2	0.4621	< 0.0001	2.04	7.0994
$Rs_{2,3}$	+2.58+0.0284*A+0.0145*B- 0.0835*C+0.0037*AB-0.0013*AC+0.0038*BC-0.2405*A^2+0.0777*B^2+0.0423*C^2	0.6185	< 0.0001	6.78	8.2538
Rt_3	+2.83+0.0181*A+0.0939*B-0.4727*C-0.0262*AB-0.0238*AC-0.0263*BC-0.0906*A^2+0.0529*B^2+0.0699*C^2	0.8396	< 0.0001	6.24	12.6168

All responses to the regression model and ANOVA reports were found within the limit shown in Table 2. %CV - Percentage coefficient of variation. The trivial terms *(p>0.05)* were terminated from the model through a backward termination procedure to acquire an uncomplicated and rational model. Therefore, R^2 values always reduce, although a regressor variable is terminated from a regression model; in statistical modeling, the adjusted R^2, which withdraws the number of regressor variables into a statement, is regularly chosen[45]. The adjusted R^2 Values were well within the allowable limits of $R^2 \geq 0.80$[46], which disclosed that the experimental data indicated a convenience with second-order polynomial equations. A p-value of < 0.05 was attained for all the reduced models, suggesting these models were important. The good precision value measures the signal (response) to noise (deviation) ratio. A ratio larger than 4 is advisable [47]. The ratio was in the range of 7.099 – 12.616, indicating a sufficient signal. Hence the model was important for the partition process. The coefficient of variation (C.V) evaluates the model's reliability. A general rule is that a model can be satisfactorily reliable if smaller than 10% [48]. In Table -2, the interconnection with the biggest perfect

coefficients between the fixed model was BC (+0.0038) of the $Rs_{2,3}$model. The positive interconnection in the middle of factors B and C was statistically significant (< 0.0001) for $Rs_{2,3}$. This work disclosed that converting the buffer pH from less to more report resulted in a fast decrease in the resolution of Indapamide and Amlodipine besylate in the less and more flow rate levels (mL/min). Additionally, the buffer pH had to be above level to decrease the run time. To acquire a finer interpretation of the outcome, the predicted models were shown in the form of perturbation plots and 3D response surface plots (Fig. 4, 5).

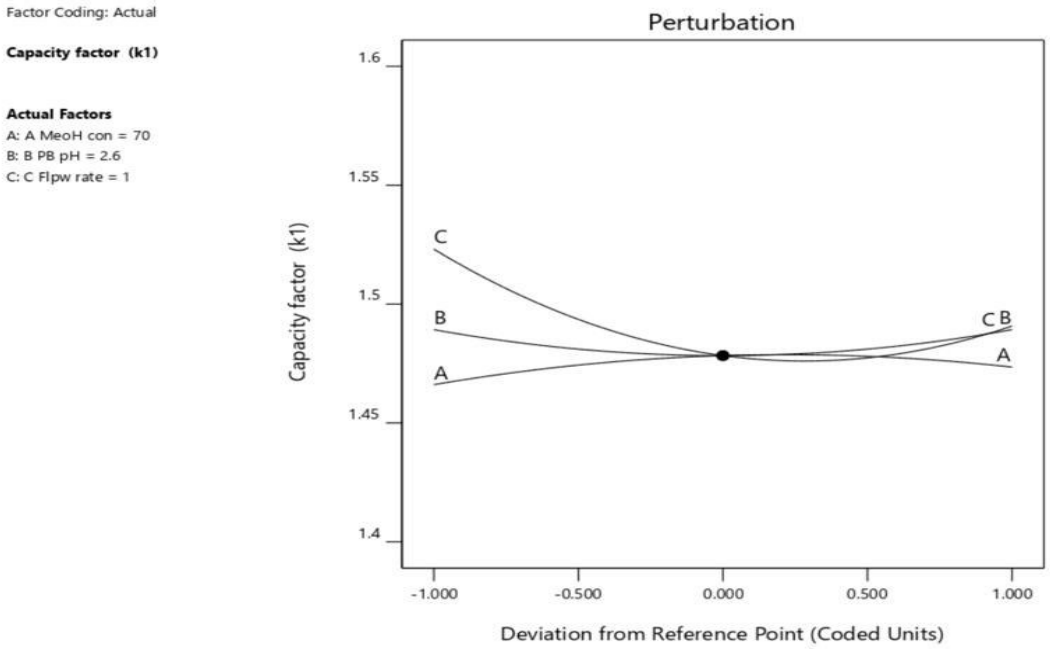

Fig 4 (a)

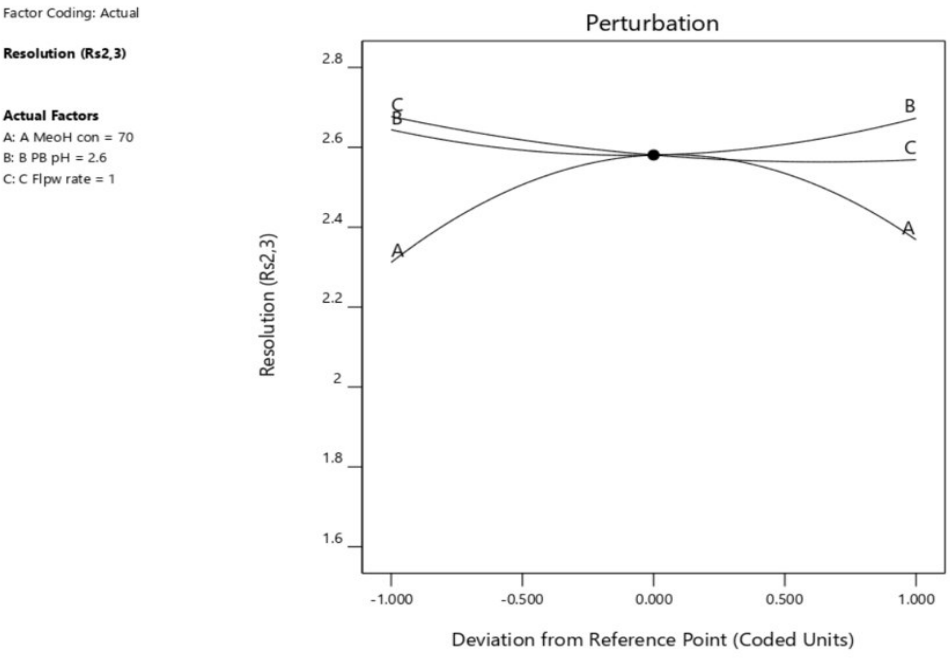

Fig 4 (b)

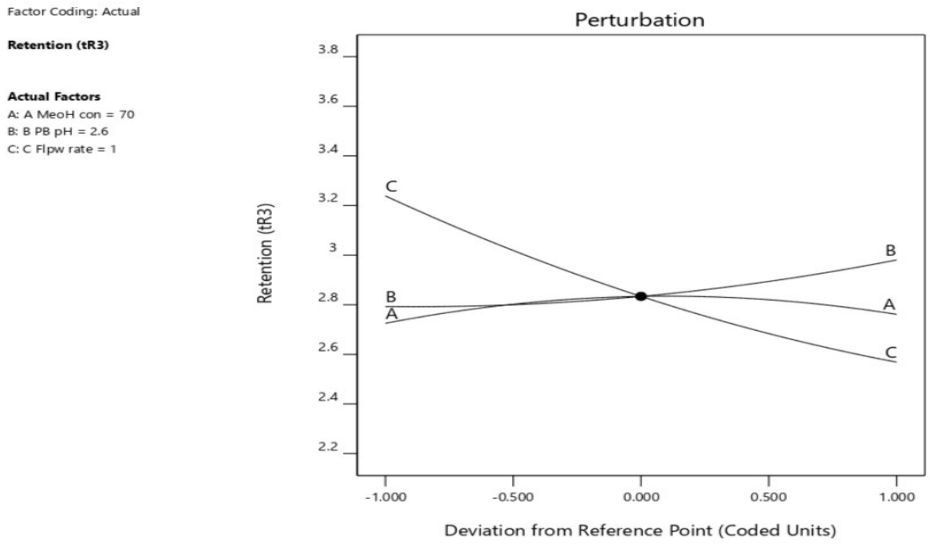

Fig 4 (c)

Fig 4: Perturbation plot for the result of the constructive ofthe study factors on the A, B, and C Responses (a) Capacity Factor k_1, (b) Resolution between peak 2 and 3 $Rs_{2,3}$, (c) Retention time tR_3 where A is the methanol concentration, B is the Phosphate buffer pH, C is the Flow rate

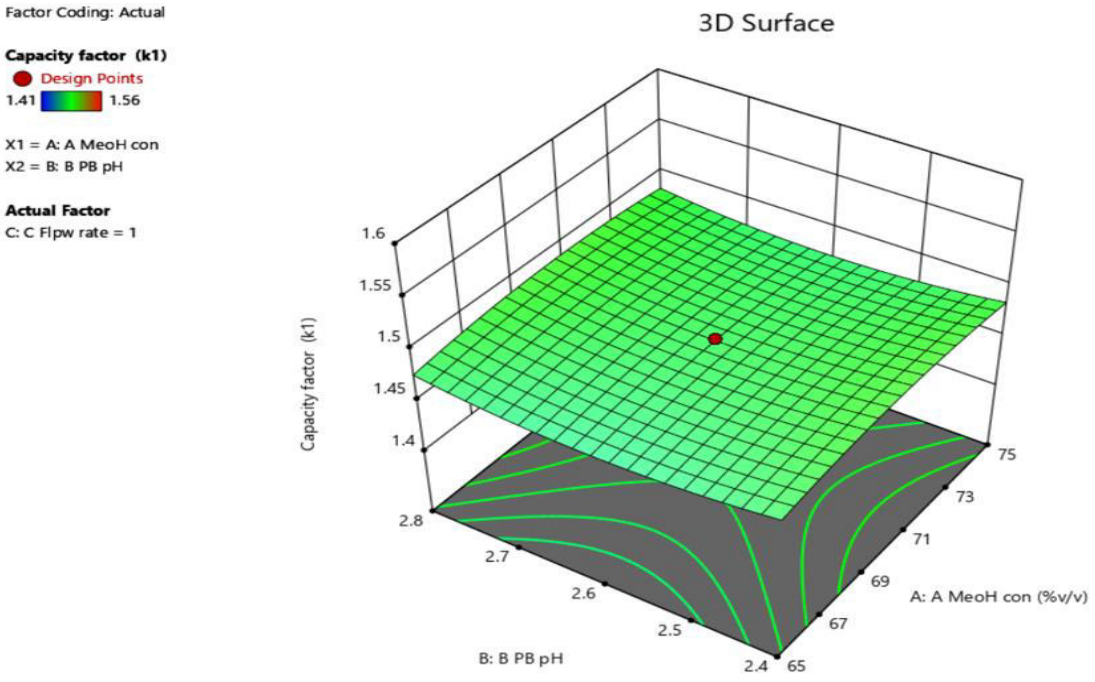

Fig 5 (a)

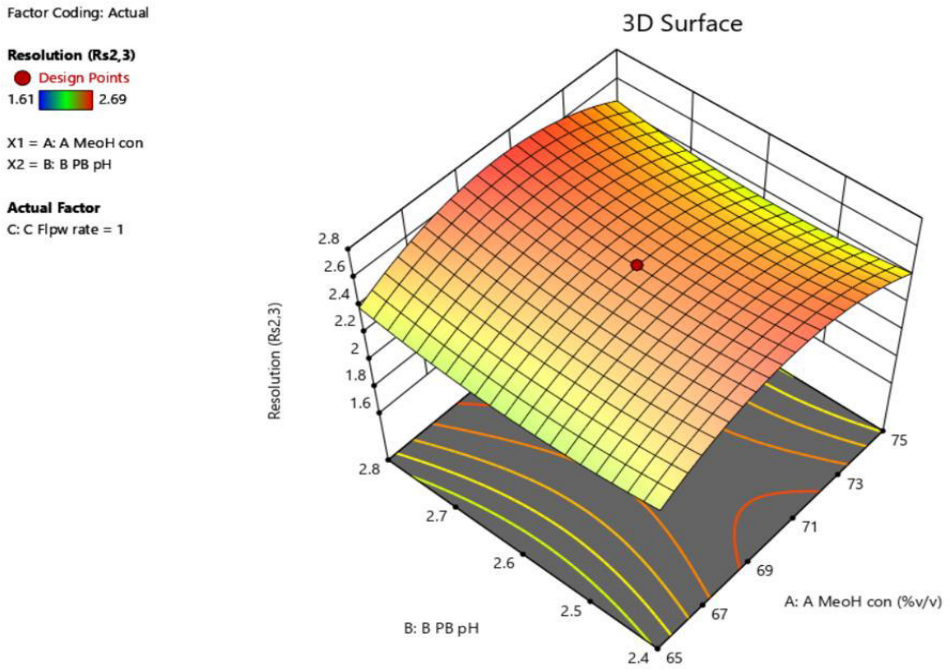

Fig 5 (b)

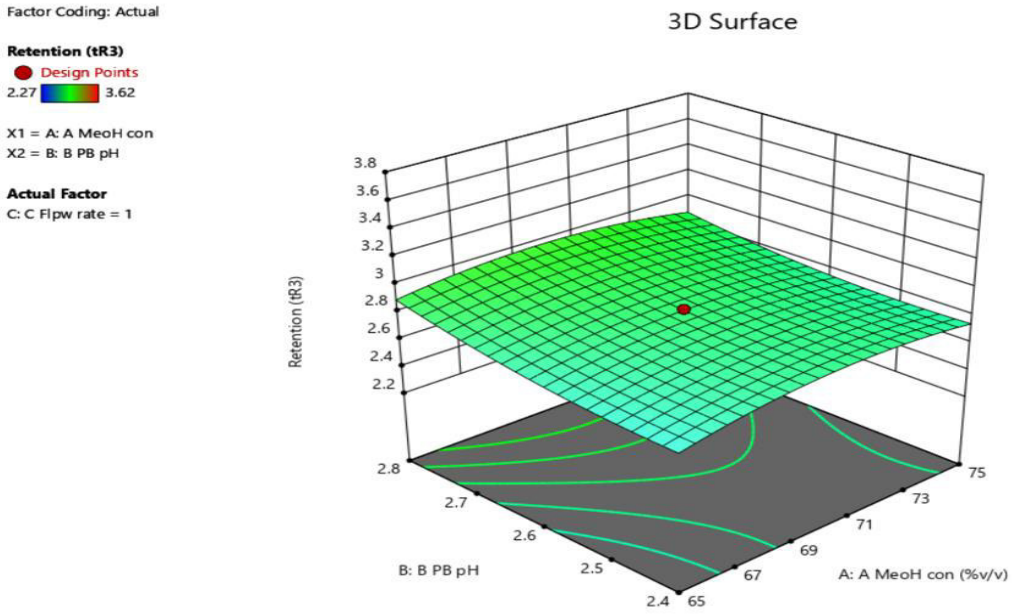

Fig5 (c)

Fig. 5: Response surface plot (3D plot) for the relationship effect of the critical factors on (a) Capacity Factor k_1, (b) Resolution between peak 2 and 3 $Rs_{2,3}$, (c) Retention time Rt_3.

Variables giving quadratic and interconnection terms with the biggest perfect coefficients in the fixed models were selected for the drop of the response surface plots. Perturbation plot presuming to outline views of the response surface plots where it indicated in what condition the response converts as every factor transferred from a selected standard point, with all factors carrying constant at the reference value. Phosphate buffer pH (factor B) had the most significant effect on resolution ($Rs_{2,3}$) following factor C (Flow rate). The rest of the factors had a significant effect on k_1 and Rt_3. k_1 values increased as the flow rate level increased, and k_1 values decreased as the level of phosphate buffer pH increased. The value of the resolution (Rt_3) increased with increasing levels of factor B. Analysis of the perturbation plots and response plots of optimization models revealed that factors B and C significantly affected the separation of the analytes[49]. Derringer's desirability function was selected for the global optimization of three responses and to employ different optimal conditions for the formulation analysis in the current study. The identified criteria for the optimization were resolution between the peaks, capacity factor, and elution time. Derringer's desirability function, D, is defined as the individual desirability functions' geometric mean, weighted or otherwise. The expression that defines Derringer's desirability function is:

$$D = [d_1^{p2} \times d_2^{p2} \times d_3^{p2} \times \ldots\ldots\ldots \times d_n^{pn}]^{1/n}$$

Where pi is the weight of the response, n is the number of responses, and di is the individual desirability function of each response. The desirability function (D) can take values from 0 to 1. Weights can range from 0.1 to 10. Weights less than 1 give less significance to the criteria, whereas weights more than 1 give more significance.[50] The goals for optimizing each response are shown in Table 3.

Table -3: Relation of Described and predictive values of different objective functions under optimum conditions.

Response	Lower limit	Upper limit	Criteria/Goal
k_1	1.41	1.56	Minimize
$Rs_{2,3}$	1.61	2.69	In the range
Rt_3	2.27	3.62	In the range

All Responses from lower value to higher value and goal are indicated in Table 3.

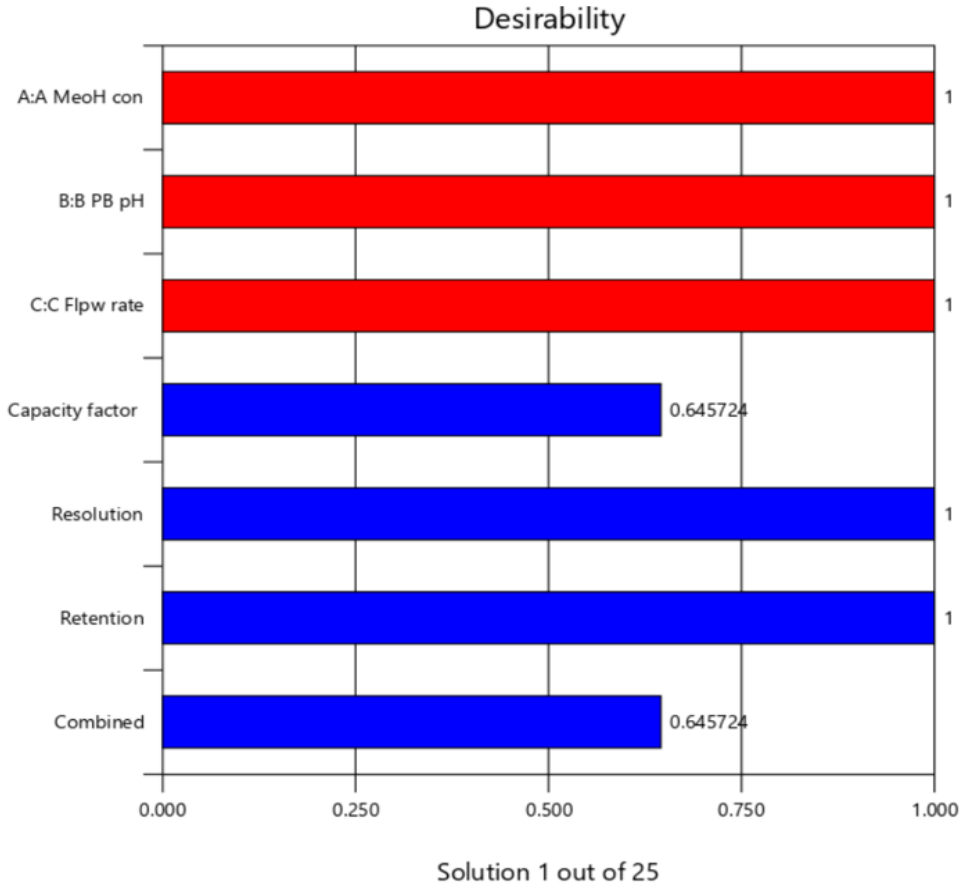

Fig. 6: Graphical Representation of global desirability function (D=0.662)

Fig. 6 showed that high desirability values (D = 0.662) were in the condition of organic solvent concentration (Methanol) of 65%, buffer pH of 2.6, and flow rate of 1.1 ml/min. Hence, the optimized assay conditions were MeOH: phosphate buffer (65:35%v/v) (pH 2.6) as mobile phase at a 1.1 ml/min flow rate. And UV detection at 215 nm. The predicted response values corresponding to the later value of D were k_1 = 1.46, $Rs_{2,3}$ = 2.484, and Rt_3 = 2.734min. The prediction efficiency of the model was confirmed by experimenting with the optimal condition, and the corresponding chromatogram was shown in Fig. 7. The observed difference between the predicted and experimental responses was found to be in good agreement, within a difference of 5.0%, was shown in Table 4.

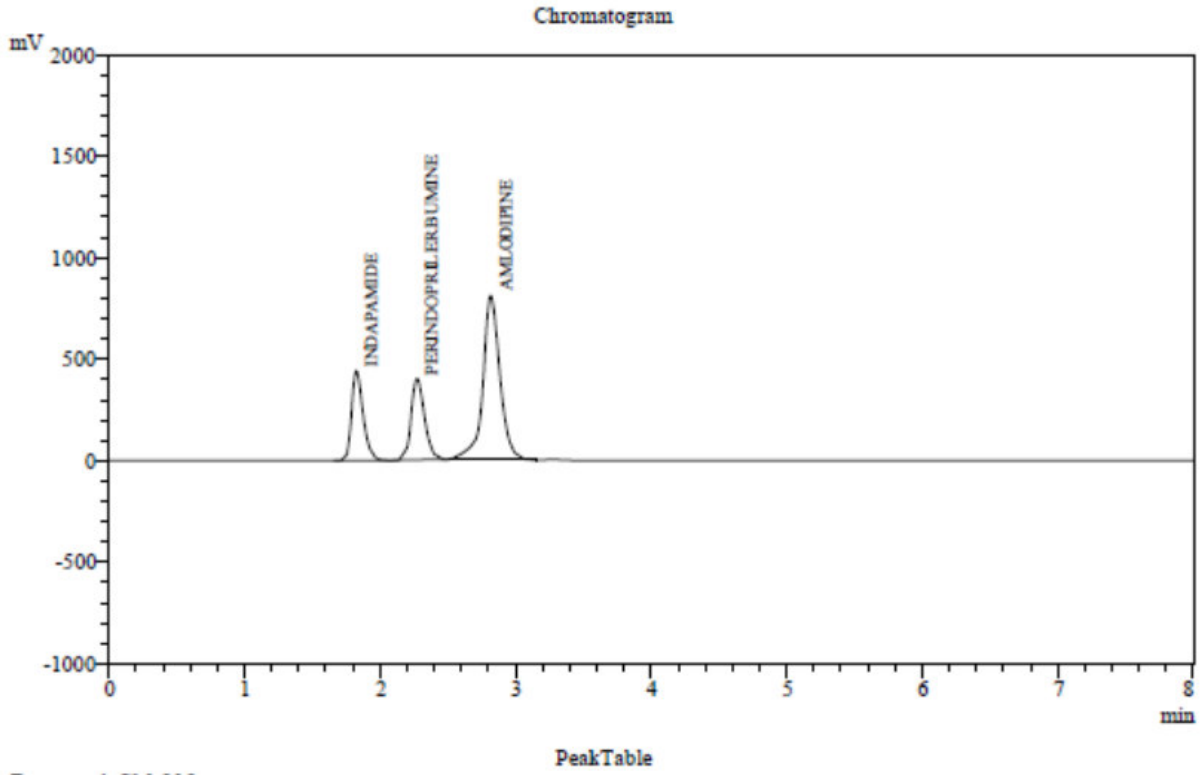

Fig. 7: Optimized Chromatogram for Indapamide, Perindopril erbumine, and Amlodipine besylate. Optimized assay conditions were MeOH: phosphate buffer (65:35%v/v) (pH 2.6) as mobile phase at a 1.1 ml/min flow rate. And UV detection at 215 nm.

Table- 4: Comparison of Experimental and Predictive Values of Different Functions under Optimal Conditions

Optimum conditions	ACN (%v/v)	Buffer pH	Flow rate (ml/min)	k1	Rs2,3	tR3
Predictive	65.00	2.6	1.1	1.46	2.484	2.735
Experimental	65.00	2.6	1.1	1.48	2.591	2.840
Average error				1.36	4.6	3.6
Desirability value (D) =0.662						

Predictive values are applied to the experimental part. The average error was obtained within 6% for all responses. The Desirability value was found to be within the limit (less than 1)

4.1. Method Validation

RP-HPLC method was optimized by using QbD and validated according to the ICH guidelines (Q2A [51], Q2B[52]).

4.1.1. System suitability parameters

System suitability test provides the added assurance that, on a specific occasion, the method gives accurate and precise results. The results of each system suitability test are compared with defined acceptance criteria, and if they pass, the method is deemed satisfactory on that occasion. Acceptance criteria for system suitability were asymmetry factor should not be more than 2.0, theoretical plates should not be less than 2000, and % RSD of peak area should not exceed 2.0. All variation parameters results were within the acceptance criteria mentioned above. The system suitability data are shown in Table 5.

Table- 5: Data for System Suitability Parameters

Injections	Name of the analyte	Conc (µg/ml)	Rt (min)	Area (μV^2 sec)	USP plate count	Resolution	Tailing factor
1	Indapamide	25	1.824	2610206	1879.558		1.46
2			1.825	2608590	1830.232		1.48
1	Perindopril Erbumine	80	2.271	7105314	2234.565	2.48	1.32
2			2.271	2715073	2287.222	2.44	1.34
1	Amlodipine	100	2.817	7100983	2431.935	2.59	0.97

	Besylate					
2		2.818	7100139	2486.877	2.55	0.99

System suitability parameters reports were shown within the acceptance limit per USP guidelines.

4.1.2. Linearity range

The linearity of an analytical method is the potential to obtain test reports that are directly proportional to the analyte concentration in samples within a given range. The linearity ranges between 10 and 40, 32 and 128, and 40 and 160 µg / mL for indapamide, Perindopril erbumine, and Amlodipine Besylate. The calibration curve was constructed using between concentrations versus the peak area of the analytes. Linear curves were observed for all drugs. Good linearity was validated by the high correlation coefficient value (r^2 = 0.9994). The linearity ranges of the reported UV spectroscopy method were more when compared to the developed method. Hence, the developed method can be applied to estimate analytes when the least amount of drugs is required.

4.1.3. Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQs) for indapamide, Perindopril erbumine, and Amlodipine Besylate are 0.0020, 0.0085, 0.029 µg/ml, and 0.0063, 0.026, 0.089 µg/ml. The LOD and LOQ value for the reported RP-HPLC[53] method was more when compared to the developed method. The detection limit and Quantitation limit values were very low, indicating the method's sensitivity. Hence, the developed method was more sensitive to compare the reported method.

4.1.4. Quantification of Formulation

An assay (content estimation) was performed to determine the purity of Indapamide, Perindopril erbumine, and Amlodipine Besylate in tablet formulation. The nominal concentration from the calibration curve was selected, and Indapamide, Perindopril erbumine and Amlodipine Besylate were quantified. The tablet formulation TRIPLIXAM was selected for analysis, and the percentage purity of analytes in the formulation ranged from 99.09 to 100.96%. The % RSD values were 0.3626, 0.6117, and 0.3867 for Indapamide, Perindopril erbumine, and Amlodipine besylate, respectively.

4.1.5. Precision

The precision data represented no considerable variation in the measured response which demonstrated that the method was repeatable with the % RSD value below 0.5 for all analytes, which met the acceptance limit (acceptance criteria – not more than 2 %). The %RSD value for precision was 0.3472, 0.4271, and 0.3592 for indapamide, Perindopril erbumine, and Amlodipine Besylate. This indicated that the developed method had good precision with repeatability.

4.1.6. Accuracy

Accuracy implies the intimacy of acceptance connecting the detected and obtained recommendation values. The accuracy data were summarized in Table- 6. Different concentrations of analytes explain that the percent recovery ranged between 99.18, 99.37%, and 99.58%. The percentage coefficient of variation value was found to be less than 2%. Based on the results, the developed method was accurate.

4.1.7. Robustness

The robustness study indicated that the factors selected remained unaffected by small flow rate variations, the organic composition of the mobile phase, and wavelength. The system suitability parameters results were within the limit. Hence the method was robust [3854].

4.1.8. Ruggedness

Ruggedness measures the reproducibility of test results under normal, expected operational conditions from analyst to analyst[54]. The percentage RSD value for analyst I was found to be 0.973, 0.8307, and 0.9034 % for Indapamide, Perindopril erbumine, and Amlodipine besylate, respectively. The percentage RSD value for analyst II was found to be 0.7161, 1.013, and 1.1508 % for IND, PER, and AML, respectively. Ruggedness results are shown in Table -7.

Table -6: Validation parameter report

Parameters	Indapamide	Perindopril Erbumine	Amlodipine Besylate
Range (µg/ml)	10-40	32-128	40-160
$y = mx + cr^2$	y = 110453x + 2481.6	y = 34330x + 3015.2	y = 71935x + 20354
Slope (m)	0.9999	0.9999	0.9999
Intercept (c)	110453	34330	71935
LOD (µg/ml)	2481.6	3015.2	20354
LOQ(µg/ml)	0.0020	0.0085	0.029
Precision (% RSD)	0.0063	0.026	0.089
Accuracy (%)	0.3472	0.4271	0.3592
Assay (%)	99.18	99.37	99.58
	99.52	99.59	100.96

Table -7: Data for Ruggedness Study

Compound	Different conditions	Average Percentage %	SD	(%) RSD
Indapamide	Analyst-I	99.52	0.9796	0.9731
Perindopril Erbumine		100.59	0.8281	0.8307
Amlodipine Besylate		98.96	0.9014	0.9034

Indapamide	Analyst-II	101.48	0.7158	0.7161
Perindopril Erbumine		99.62	1.0163	1.0131
Amlodipine Besylate		99.84	1.1490	1.1508

All validation parameters reports were within the acceptance criteria. The detection and quantification limits were much lower than the already reported method. Ruggedness data (%RSD) value indicates within the limit (less than 2%)

5. CONCLUSION

The experimental design explains the search for the key components in the HPLC method, including mobile phase composition, Buffer pH, and flow rate at their three different levels. Factors and responses to their interrelationship were studied and optimized using a central composite design. Now a better understanding of the factors affecting chromatographic separation in the ability of the methods to meet their intended purposes was done. All the validated parameters were found within the acceptance criteria. The validated method was linear, precise, specific, and accurate.The Experimental automated design (QbD) method development approach using the Design Expert software has provided better performance in less time compared to manual method development. The statistical data analysis indicates that the method is reproducible, selective, and accurate. This method will be used further for routine pharmaceutical industry quality control analysis.

6. ACKNOWLEDGEMENT

The authors are grateful to Adhiparasakthi College of Pharmacy, Melmaruvathur, Tamilnadu. The facilities provided to complete the Research work completely.

7. AUTHORS CONTRIBUTION STATEMENT

Dr. Thomas Sudha conceived and designed the study and Mr. Arumugam Sarveswaran prepared the data and the original draft. Dr. Palani Kumar Nallasivanand Mrs. Munnusamy Vijayakumar Saranya discussed the experimental section and determined the data. All authors discuss the data analysis and valuable inputs to designing the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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