



HPLC method development for determination of metoprolol, telmisartan and cilnidipine simultaneously using response surface design

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ABSTRACT

The development of an HPLC method for determination of Metoprolol, Telmisartan and Cilnidipine simultaneously in additional dosage was not published or reported up till date. The main aim of our study was to design a simultaneous and multiple response optimizations using the Derringer's desirability function in order to estimate Metoprolol, Telmisartan and Cilnidipine in pharmaceutical and bulk drug dosage form by HPLC method with experiment central composite design (CCD) protocol for the quantitative methods analysis and also for validation of the procedure that is developed as per ICH regulations. An innovative RP-HPLC method had been designed for the estimation of Metoprolol, Telmisartan and Cilnidipine simultaneously in formulation using central composite design. Three factors were investigated and determined as significant when compared to the interaction and quadratic effect of the samples that CCD along with the response of the surface methodology. The developed method produced a good resolution of the drugs with a very short run time of 7.5 min. It was also validated according to ICH guidelines. It was recognized as novel and simple method that is accurate and cost-effective. So the proposed method fits best in the assaying routine of Metoprolol, Telmisartan and Cilnidipine in any formulations produced by quality control laboratories.



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INTRODUCTION

Metoprolol succinate (MET) Figure 1a is a cardio drug. It is used to treat hypertension and various cardiovascular disorders. It is chemi-

cally known as (\pm) 1- (isopropyl amino)-3-[p-(2-methoxyethyl) phenoxy]- 2-propanol succinate (2:1) (Gad, 2014; Siripuram et al., 2010). Cilnidipine Figure 1b is chemically 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine carboxylic acid-2-methoxy ethyl-(2E)-3-phenyl-propenylester (Kim et al., 2006). Cilnidipine exhibits its action by blocking the calcium channels that are incoming which are present on the L-type of receptors on the blood vessels (Uneyama et al., 1999). Telmisartan Figure 1c is chemically 2-(4-([4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)]-2-propyl-1H-1,3-benzodiazol-1-yl] methyl) phenyl) benzoic acid (Bakheit et al., 2015). Telmisartan is used to manage hypertension and acts on the angiotensin receptor of type II y antagonizing the receptors. Literature survey states that metoprolol and telmisartan or metoprolol succinate and cilnidipine or

cilnidipine and olmesetran are legal in IP, USP and BP isolatedly, however, a combination of Metoprolol, Telmisartan and Cilnidipine is not made official in any of the Pharmacopoeias. Numerous analytical methods like HPLC (Kardani *et al.*, 2013), Stability indicating HPLC (Rupareliya *et al.*, 2013), Spectrophotometry (Haripriya *et al.*, 2013), HPTLC (Desai *et al.*, 2016) and stability-indicating HPTLC (Santosh *et al.*, 2015) procedures were mentioned in the literature for the estimation of Metoprolol, Telmisartan and Cilnidipine single drugs and as combinations too with other moieties in a dosage form. Conversely, the development of an HPLC method for simultaneous determination of Metoprolol, Telmisartan and Cilnidipine in additional dosage form has not been published or reported up till date. The objective behind of our current study was to design simultaneous multiple response optimizations using the Derringer's desirability function for estimation Metoprolol, Telmisartan and Cilnidipine in pharmaceutical and bulk dosage form by HPLC method using experiment central composite design (CCD) protocol for the quantitative methods analysis and also for validation of the method that is developed as per ICH guidelines.

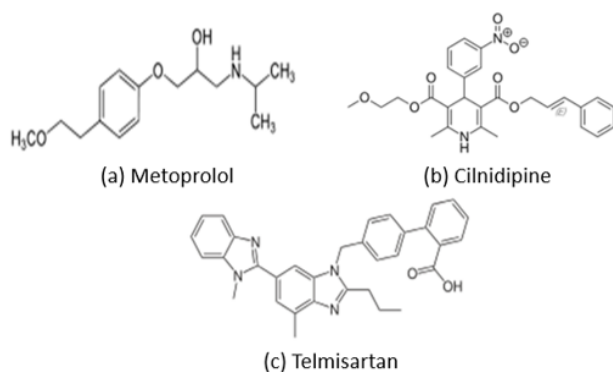


Figure 1: Structure for Analytes

MATERIALS AND METHODS

Reagents

Pharmaceutical pure analytical grade samples of Metoprolol, Telmisartan and Cilnidipine were collected from the company called Dr. Reddy's, Hyderabad, which were given as gift samples without any further purification process. A combination of Metoprolol, Cilnidipine and Telmesartan tablet formulations (Arbitel-Trio 25mg) was procured from the local market. HPLC graded methanol, water, Acetonitrile and other chemicals like Orthophosphoric acid buffered in the AR grade were bought from the Merck chemicals Pvt Ltd, India.

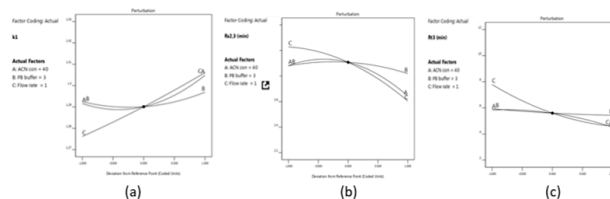


Figure 2: Perturbation plots for (a) K_1 (b) $R_{s2,3}$ (c) R_{t3}

Instrumentation & Chromatography Conditions

Analysis was conducted with a Shimadzu LC2010 CHT separation module equipped with LC solution software, Pump LC2010 binary and UV detector set at 270 nm. Compounds were isolated on an Intek chromasol column (250×4.6 mm) under reversed phase partition conditions. Mobile phase was Acetonitrile and phosphate buffer. Sample flow was maintained at 1ml per min and the total run time was maintained at 8mins. Samples were then injected through a rheodyne injector which has a 10 microns.

Detection and looping were carried out at the wavelength of 270 nm. Degassing was done to the mobile phase before analyzing and injecting the sample. The sonicator (Ultrasonic power cleaner sonic 420) was used to degass the phase. It was then filtered through a nylon filter of 0.45 microns. The experiments were conducted with the maintenance of temperature of column at $30 \pm 5^\circ\text{C}$.

Preparation of mobile phase

650ml of phosphate buffer (pH 3.2) & 350ml of acetonitrile were added in beaker and placed in an ultrasonicator for degassing and placed in a water bath for about 5 min. It was then filtered thoroughly with a filter press under vacuum and then transferred to a volumetric flask of 1L capacity.

Preparation of working standard stock solution

Approximately 50mg of Metoprolol, 80mg of Telmisartan and 20mg of Cilnidipine were accurately weighed and then transferred to a 100ml flask. 10ml of the mobile phase was mixed to the contents and then sonicated for about 15mins. The volumes were made to 100ml with a mobile phase solvent. It was then made up to the mark to achieve the concentration of $500 \mu\text{g/ml}$ for Metoprolol, $800 \mu\text{g/ml}$ Telmisartan & $200 \mu\text{g/ml}$ Cilnidipine.

Preparation of sample solution

Ten tablets (Arbitel-Trio) were weighed to the accuracy and were crushed and powdered into a fine powder. This powder was weighed that is equivalent to 40 mg of the drug and was collected into a 100ml of the volumetric beaker.

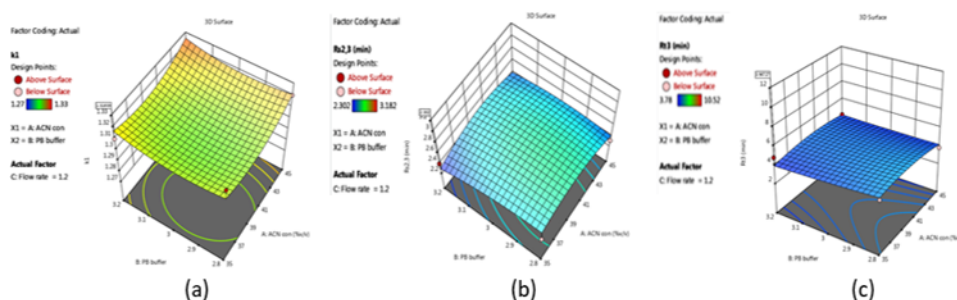


Figure 3: 3D Response Surface Plot for (a) K_1 (b) $Rs_{2,3}$ (c) Rt_3

About 50 ml of mobile phase was mixed, shook for 5 minutes and then ultrasonicated for 20 mins along with intermittent shaking. After this, the volume was finally made to the mark with 100ml of the mobile phase. 3.5 ml of the above solution was pipetted out and transferred to a 50 ml volumetric flask and made to the volume with the same. It was then filtered into the membrane with 0.45 microns filter. The final concentrations of the solutions were then made to 14 $\mu\text{g}/\text{ml}$ for Cilnidipine, 35 $\mu\text{g}/\text{ml}$ for Metoprolol and 56 $\mu\text{g}/\text{ml}$ for Telmisartan.

Experimental design

Central composite design was used in order to optimize the composition parameters and also to evaluate the main effects and interaction of the effects and quadratic effects of the parameters that are favoring the retardation factor of all the drugs. CCD is helpful for the response surface methodology and for also exploring the quadratic response in the surfaces and also to construct 2^{nd} order, polynomial models. This is also done without the need of third level factorial design experiments. Experimental designs were done to approach the users to optimize the separation of the constituents and also to help the development of the betterment of understanding of the several chromatography factors on the quality of separation of the constituents. In this research important factor of the chromatography was selected on the basis of preliminary experiments also the prior understanding of literature and the optimization by centrally composite design of the experiments. A CCD was employed for the location of the optimum flow rate of eh mobile phase and pH, the volume of the organic modifier that is used for the separation of mapping the response curve of chromatography surface (ICH, 2003). Composite Design is used to provide the three independent variables of the design and a partially factorial design that was combined with the five replicas of the central point and five axis points at the extreme levels. The 2^{nd} order model was fitted into the experimental models. The quality of the fitted models of the polynomial equations was examined based on the coefficient of the

determination of the R2 values. The position and conditions were optimum were noticed by the application of the derringers desirability of the function. The responses were simultaneously optimized.

The last stage of the prediction of the response and the designing space was from the polynomial equation. Response methodology surface is a mathematical technique for application of statistics that are valuable for the analysis of problems that were independent of the column temperature, flow rate, pH and other variables like the resolution, run time and peaking etc. this was used to the optimization of the levels of the variables in order to attain the best performance of the system. The RSM provokes the definitions of the models of quadratics that explains accurately the response of the values of the chromatography conditions followed in the experimental designs. In order to calculate the regression of the quadratic equation and each designing variable should be studied and the three levels of the variations consequently. The CCD was applied to optimize the study (Ermer and John, 2005).

Selectivity

The selective nature of the method is often depicted with a well sharp resolved peaks that correspond to metoprolol, telmisartan and cilnidipine. The method was checked for its specificity by checking the chromatograms comparison that is obtained for the standard drug and the formulations along with placebo. The retention time of the standard drug and the drugs of sample solution was determined as identical. The confirmation of the specificity of the selected method was done with this study.

Linearity

The linearity of the method of analysis is the capacity of the test to deliver results that are directly proportional to the analyte concentration and the sample within the specified range. About 50 mg of Metoprolol, 80 mg of Telmisartan and 20 mg of Cilnidipine were measured and are transferred into 100ml flask. In this 10ml mobile phase was mixed and ultrasonicated for dissolution.

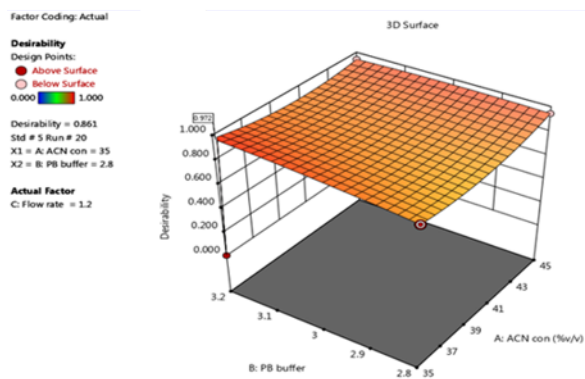


Figure 4: Global desirability response surface plot

The volume was made up to the mark. The concentrations of 500 $\mu\text{g/ml}$, 800 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$ for Metoprolol, Telmisartan and Cilnidipine respectively were obtained. The standard stock solutions were pipetted out 2.5, 3, 3.5, 4.0 and 4.5 ml separately, transferred into series of five 50 ml standard flasks and diluted with the mobile phase. The final concentration of the solutions was in the range of 25-45 $\mu\text{g/ml}$ for Metoprolol, 40-72 $\mu\text{g/ml}$ for Telmisartan and 10-18 $\mu\text{g/ml}$ for Cilnidipine. 20 μl solutions of each concentration were injected and chromatograms were recorded. The calibration curves were drawn by using the peak area versus the concentration.

Limits of detection

The lowest of the concentration present in the sample that is detectable, but it is not necessarily the quantified under the experimental conditions. The limit of the detection is most important to detect the impurities and the assay of the dosage forms that contain low drug levels and the placebos. The limit of the detections was calculated with the use of the formulae as follows.

$$\text{LOD} = 3.3 \times \text{std.dev/slope}$$

The limit of detection was used to calculate the average value of the slope and the standard intercept deviation.

Limits of quantification

This is the least concentration present in a sample that can be detected and quantified. LOQ was calculated by using the following formula.

$$\text{LOQ} = 10 \times \text{std.dev/slope}$$

Preparation of calibration curve from the serial dilution of the standard was repeated for three times. The limit of quantification that was calculated with use of slope and standard deviation of the intercept.

Content estimation

35 $\mu\text{g/ml}$ of Metoprolol, 56 $\mu\text{g/ml}$ of Telmisartan and 14 $\mu\text{g/ml}$ of Cilnidipine standard drug and the solution that contained sample that was prepared & 20 microns of eh standard and the sample solutions that were injected and the chromatogram were noted. The % purity of the samples was calculated. The percentage purity of analytes was calculated.

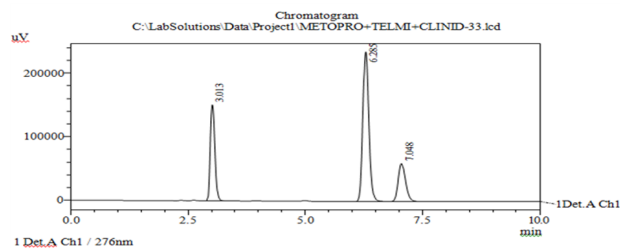


Figure 5: Optimal conditions corresponding Chromatogram

Precision

The precision of this analytical method of eh degree of the agreements that are among the individual tests results that are obtained when the methods are applied to multiple samples of the homogenous sample in the same day. Aliquots of standard stock solution of Metoprolol, Telmisartan and Cilnidipine (3.5 ml of 500 $\mu\text{g/ml}$ of Metoprolol, 3.5 ml of 800 $\mu\text{g/ml}$ of Telmisartan and 3.5 ml of 200 $\mu\text{g/ml}$ of Cilnidipine) were transferred into a 10 ml of the standard flask and made up to the specified mark using mobile phase. 20 μl of the solution was injected and the chromatograms were noted. This procedure was again repeated for five times in one day. The peaks area was measured and the % RSD was calculated.

Accuracy

Accuracy of a procedure was defined as the closeness of the values that fall in the range of accepted values of the reference and values found. The accuracy of the method was analyzed by hiking the sample with a reference compound. It was evaluated in triplicate at the concentration levels (50%, 75% & 100%) of the target test concentrations (500 $\mu\text{g/ml}$ of Metoprolol, 800 $\mu\text{g/ml}$ of Telmisartan and 200 $\mu\text{g/ml}$ for Cilnidipine). 20 μl of the sample solutions of the concentrations were injected and the chromatograms were noted and recorded.

Robustness

Robustness of study was evaluated and studied with the effect of minute and random variations in the chromatography. The study conditions were evaluated flow rates (± 0.1 ml/min) and also the composition of the mobile phase. For each of the conditions measured concentration was injected into the chro-

Table 1: Central composite arrangements and response

Run	Space type	Factor 1A: CN Con %v/v	Factor 2B: PB buffer	Factor 3C: Flow rate ml/min	Response 1: k_1	Response 2: $Rs_{2,3}$ min	Response 3: Rt_3 min
1	Center	40	3	1	1.29	2.908	5.59
2	Center	40	3	1	1.29	2.908	5.59
5	Center	40	3	1	1.29	2.908	5.59
10	Center	40	3	1	1.29	2.908	5.59
12	Center	40	3	1	1.29	2.908	5.59
14	Center	40	3	1	1.29	2.908	5.59
4	Axial	40	3.33	1	1.32	2.515	4.67
8	Axial	31.59	3	1	1.3	2.628	5.07
9	Axial	40	3	0.66	1.27	2.87	10.52
11	Axial	40	3	1.33	1.32	2.42	4.33
15	Axial	40	2.66	1	1.29	2.964	7.27
16	Axial	48.409	3	1	1.33	2.356	4.04
3	factorial	45	2.8	1.2	1.32	2.302	3.78
6	factorial	35	3.2	1.2	1.31	2.399	4.83
7	factorial	35	3.2	0.8	1.28	3.103	8.32
13	factorial	45	2.8	0.8	1.3	2.445	5.25
17	factorial	45	3.2	0.8	1.29	2.804	6.11
18	factorial	35	2.8	0.8	1.28	3.182	8.97
19	factorial	45	3.2	1.2	1.32	2.33	4.17
20	factorial	35	2.8	1.2	1.32	2.364	4.48

matogram systems and the data was recorded. The suitability parameters were checked for the system.

Ruggedness

The reproducibility of the study of the test resulted by the proposed study method of the analytes was analyzed by the samples under the following study using a variety of the conditions with varying analyst and varying instruments.

RESULTS AND DISCUSSION

Analytes are polar in nature. The reverse phase of HPLC was used as a preferable way. Column chemistry (C_{18}), solvent type (acetonitrile or methanol), solvent strength and flow rate were then varied to determine the best chromatographic conditions that give quality separation. Mobile phase conditions were optimized such that the first eluting component does not interfere with the peaks of solvent and excipient. Other criteria like analysis time, appropriate k range ($1 < k < 10$) for eluted peaks, tailing factor, assay sensitivity and noise were also considered. Intek chromasol C_{18} column (250x4.6 mm, 5 μ m) and mobile phase consisted of acetonitrile: phosphate buffer (pH 3.0) were tried to examine initial separation conditions.

Before start of the optimization of procedure, it is very crucial to investigate the curvature using factorial design using central points of ANOVA generation 2^K factorial design displayed that curve is significant based on responses (k_1 , $Rs_{2,3}$, tR_3) since p -value was less than 0.05. Quadratic model that is implied should be taken into account the separation. To achieve the 2^{nd} order predictive modeling, the central composite design type response was employed. CCD was considered to use flexibility and it could also be applied for optimizing HPLC separation to gain better knowledge in factors. Selection of factors to optimize on basis of preliminary study and previous knowledge of literature along with instrumental limits, is to be considered. From preliminary procedures, a C_{18} column stationary phase and mobile phase contains acetonitrile: phosphate buffer (pH 3.0) was employed.

Volume of phosphate buffer inside the mobile phase was kept constant at 40% and acetonitrile volume is only altered. The flow rates were known to cause minute changes in the HPLC analysis. So the major factors for the perfect process are the concentration of acetonitrile, pH of the buffer and also flow rates. Table 1 showed the levels of each factor studied for finding out the optimum values and responses.

Table 2: Reduced response surface models and statistics that are resultant of ANOVA

Response	Model of regression	Modified R2	Modular P-value	(%) C.V	Required precision
k1	+1.29-0.006*A+0.002*B+0.014*C+0.011*AB-0.002*AC+0.001*BC-0.008*A ² +0.004*B ² +0.001*C ²	0.8028	< 0.0001	2.58	10.7160
Rs2,3	+2.61-0.118*A-0.030*B-0.212*C+0.053*AB+0.113*AC-0.027*BC-0.145*A ² -0.057*B ² -0.091*C ²	0.8554	< 0.0001	5.24	8.1893
Rt3	+5.60-0.66*A-0.250*B-1.60*C+0.193*AB+0.571*AC+0.066*BC-0.422*A ² +0.077*B ² +0.592*C ²	0.8386	< 0.0001	12.17	13.4349

Table 3: The criteria for the optimum individual response

Response	Lesser value	Higher value	Criteria/Goal
k1	1.27	1.33	Is in range
Rs2,3	2.302	3.182	Is in range
Rt3	3.78	10.52	minimize

Table 4: Comparative response of the experimental and predictive procedure values of various functions that are under the optimal conditions

Optimal conditions	ACN (%v/v)	Buffers (pH)	Flow rates (ml/min)	k ₁	Rs _{2,3}	tR ₃
Predictive	35.00	3.20	1.3	1.35	3.33	7.12
Experimental	35.00	3.20	1.3	1.31	3.17	7.04
Average error	-	-	-	2.96	5.04	1.12

Desirability value (D) =0.972

Table 5: Validation Parameters

Parameters	Metoprolol	Telmisartan	Cilnidipine
Range (μgml^{-1})	25-45	40-72	20-36
Y=mx + c	y = 27460x + 5764	y = 34511x + 36991	y = 20720x + 1583
r ²	0.9994	0.9997	0.9992
Slope (m)	27460	34511	20720
Intercept (c)	5764	36991	1583
LOD (μgml^{-1})	0.0109	0.0420	0.0083
LOQ (μgml^{-1})	0.0333	0.1273	0.0253
Accuracy (%)	101.06	101.06	101.06
Precision (%RSD)	0.3226	1.899	0.6021
Ruggedness	Analyst-I	99.10	98.48
	Analyst-II	100.07	101.42

In Table 1 the ranges of each factor used were acetonitrile concentration (35-45 %v/v), buffer pH (2.8-3.2) and flow rate (0.8-1.2 ml/min). As response variables, the capacity factor for the 1st eluted peak metoprolol (k_1), the resolution between two peaks telmisartan and cilnidipine ($Rs_{2,3}$), the retention time of the last peak cilnidipine (tR_3) were selected. Three factors like quadratic, cross terms and linear terms were incorporated for factorial design and models are represented as follows.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_{12} + \beta_{22} X_{22} + \beta_{33} X_{32}$$

where Y is considered as the response to be taken as a model, β is considered as the regression coefficient and X_1 , X_2 and X_3 represent factors A, B and C.

Insignificance of the terms of the study was avoided and eliminated using the model through the regression elimination procedure to achieve a simple and true model. As the R² value reduces and the regresses variables are eliminated from the model, the statistical modeling of the R² value was moderated. This takes a large number of variables into account and is usually detected (Parajo *et al.*, 1992). The R² value that is adjusted for the study was within the normal limits of the acceptance of the R² greater than 0.8 (Lundstedt *et al.*, 1998), this revealed that the data that were achieved displayed a very good fitting in the 2nd order equations in the polynomial form. For all the above models that are reduced p-value lower than 0.05 were obtained by implying the models that were significantly higher. The adequacy of the precision of the signal measure was ratio to the noise measured. The ration was greater than that of 4 was considered as desired (Beg *et al.*, 2003). The ration was estimated as ranging from 8.1893 to 13.4349 with an adequate signal and hence it is called a model that is significant for the separation of the process. The coefficient of the variation CV was a measure of the reproducibility of the model and is a good rule of the designed model that is reasonably considered as reproducible if it's found less than that of 10%. The interaction terms along with the largest of the coefficient in the model that is fitted were AC of Rt_3 model which was given in Table 2. Interaction between the A and C were positive and significant, statistically at $P < 0.0001$.

The study focused that changing the concentration of acetonitrile from low to high resulted in the retention time of cilnidipine at the flow rate of low and high levels. Further, at a low level of acetonitrile concentration (factor A), an increase in flow rate resulted in a marginal decrease in the retention time. When the concentration was set at its lowest level,

the flow rate had to be the highest level to shorten the run time. The presence of the interactions in the system emphasizes the need for the carrying out of the active multi factor studies to optimize the chromatography conditions for separation. Thus to gain a better understanding of the results of the predicted analysis were showed and represented in the perturbation of Figure 2 and also 3D surface response curves were represented in Figure 3. Variables that are giving the quadratic and the interaction of the terms that has the largest of the absolute of the coefficients in the best fitted models were also chosen from the axes of the response of surface plots. The perturbation of the plots also provides the silhouette of the views that are changeable during the axes of the responses in the surface of plots. Here it shows the response changes that were different for different factors that move from a different path chosen from a reference path and also the factors that are held as constants from the value of the reference. The curve shape represents the indication of the sensitiveness of the response. The slop is higher of the peak indicates the sensitivity of the study and the specific factor. Figure 2c shows that acetonitrile concentration (factor A) had the most important effect on retention time (tR_3) following factor C. Rest of the factors had a significant effect on K_1 and $Rs_{2,3}$ Figure 2a showed that K_1 values increased as the level of buffer pH increased and that K_1 values decreased as the level of acetonitrile concentration increased. Value of the resolution ($Rs_{2,3}$) increased with rising levels of A and C. Derringer's desirability function was employed for global optimization of three responses and to select different optimal conditions for the analysis of formulation in the current study. The noticed criteria for optimizing were resolution in between the peaks, elution time and capacity factor. The geometric mean, the Derringer's desirability function weighed of the individual functions of desirability. The equation that defines the Derringer's desirability function is:

$$D = [d_1^{p^2} \times d_2^{p^2} \times d_3^{p^2} \times \dots \times d_n^{p^n}]^{1/n}$$

The data of pi is taken as the weight of the response curve. N is considered as the number of responses and the di is indicated as the desirability function of the individual response. Desirability of function is the values that are taken from 0-1 and weights were taken that ranges from 0.1 to 10. The weights that are less than the 1 means that there is little importance of the goal and which are higher than 1 means there is higher importance to the goal of study. The basic criteria for the optimization of the individual in each response were given in Table 3. In criteria, the curve responses tR_3 were minimal for shortening the analyzing time and $Rs_{2,3}$ were in range so

as to allow the baseline separation of telmisartan and cilnidipine. For separating the first eluting peak metoprolol from the solvent front, k_1 was in range. By following the normal condition for restrictions above the optimum procedure was continued for the analysis. The response of the surface of the graph was obtained as the global function of desirability and was resented in Figure 4. It is evident that the coordinates that are set produced high desirability values, as shown in Figure 4 were acetonitrile of 35.0%, buffer pH of 3.2 and flow of 1.3 ml/min. The optimized assay conditions were acetonitrile: phosphate buffer (35.0:65 %v/v) (pH 3.2, buffer strength 0.05M) as a mobile phase with a flow rate of 1.3 ml/min. And UV-detection at 276 nm. The response values that were predicted corresponding to the later value of D were $k_1 = 1.35$, $Rs_{2,3} = 3.33$ and $tR_3 = 7.5$ min. The efficiency of the prediction of the model was confirmed after performing the analysis under normal conditions and the chromatograms are given in Figure 5. The differences that are observed are predicted in the experimental responses found in the good agreement in the difference of 2% which are given in Table 4.

Method validation

There are several analytical tests that are performed for getting the satisfactory separation of resolution of study. Metoprolol, Telmisartan and Cilnidipine were tested in different mobile phase systems and along with various kinds of buffers and also organic solvents using various kinds of columns. Desired mobile phase, as found with acetonitrile and phosphate buffer solution. The mobile phase that had given the satisfactory and very good resolution of drugs, Metoprolol, Telmisartan and Cilnidipine. The increase in the pH or decrease in the pH, the mobile phase of the study did not show any significant changes in the retention time of each of the analyte. The retention time R_t of Metoprolol, Telmisartan and Cilnidipine present on the column was investigated for the flow rate of 1ml per min. The injection volume of the sample was about 10 microns. The retention time R_t of the standard solution of drugs and samples of Metoprolol, Telmisartan and Cilnidipine gave satisfactory results and resolution. The work had a main focus on the optimization of the columns and the conditions that are simple and rapid for evaluation in a cost-effective manner and very rapid in results. This includes the section of the reasonable conditions of the coloums mobiles phases and other parameters. The type of solvent, the strength of the solvent in the mobile phases and the pH of the buffers and the detection wavelength were also determined in the chromatography parameters that give the best and clear separation of

the constituents (ICH Expert Working Group, 2003).

Linearity

Parameter of linearity of the study was investigated after analyzing the series of different concentrations of the compounds. Five concentrations of the samples were selected and that ranged from 25-45, 40-72 and 10-36 μgml^{-1} Metoprolol, Telmisartan and Cilnidipine. Individual concentrations were tested repeatedly for about three times. Linearity of curve of the calibration and adherence of study was according to the beer lambart law that was validated with the help of a high value of the correlation of the coefficient 0.999 towards all the established drugs.

Limits of the Detection and quantitation

Following the ICH guidelines, instant approach of deviations are based on response of slope of drugs to determination of detection and quantification of limits. Values that are established in theory are analyzed and compared to practical values of method and limits were determined as 0.0109, 0.0420 and 0.0083 μgml^{-1} of metoprolol, telmisartan and cilnidipine and quantitation limit of 0.0333, 0.1273 and 0.0253 μgml^{-1} of metoprolol, telmisartan and cilnidipine.

Content estimation

Assay of the samples was performed to investigate the purity of the samples of Metoprolol, Telmisartan and Cilnidipine in a tablet dosage form. The formal concentration of the tablet formulation was selected for the study and determination of the percentage of the purity that is present in the formulation that was found as ranging from 99.59 to 99.97%. The % RSD values were found to be 0.3302, 0.8533 and 0.5402 for Metoprolol, Telmisartan and Cilnidipine.

Precision

Repeatability of the study was investigated for the level of every compound and the analysis of it was described under the normal experimental condition of the study. The relative mean of the deviation of the samples for Metoprolol, Telmisartan and Cilnidipine were found to be 0.3226, 1.899 and 0.6021.

Accuracy

Recovery of the samples of the drugs of Metoprolol, Telmisartan and Cilnidipine were 101.06, 100.59 and 100.60%. For all the issues in the results showed a food and accurate result of the methods. Contrarily the excipients of the pharmaceutical dosage forms that do not intervene with the analysis of the compounds that are present in formulation.

Robustness

Values of the robustness of investigation indicate that the factors were selected that are remained not

effected due to minor variations of the flow rates of solvents and composition of mobile phases. Suitability of system of analysis results shown in this is limit and method is declared as robust.

Ruggedness

Data for ruggedness is a typical measure of reproducible results of the tests which are normal under the normal conditions. Results were expected and normal for these operational conditions in the laboratory from the analyst. The % of the RSD of the analyst I was also determined to as 0.76, 0.21 and 0.90% for Metoprolol, Telmisartan and Cilnidipine. The % of the RSD of the analyst II was also determined to as 0.69, 1.18 and 0.63% for Metoprolol, Telmisartan and Cilnidipine. Method validation parameter reports were shown Table 5.

CONCLUSIONS

An innovative RP-HPLC method was designed to estimate Metoprolol, Telmisartan and Cilnidipine simultaneously in a marketed formula using central composite design. The regression of the multivariate study was also successful in employing to determine the screening of the major effects of the factors that are significant. The effect of the resolution and color efficacy and tailing of the important peaks was determined. The three factors were investigated and determined as significant when compared to the interactions and quadratic effects of the samples that CCD along with the response of the surface methodology. The developed method produced a good resolution of the drugs with a very short run time of 7.5min. It was also validated according to the ICH guidelines. It was recognized as a novel and simple method that is accurate and cost-effective. So the proposed method fits best in the assaying routine of Metoprolol, Telmisartan and Cilnidipine in any formulations produced by quality control laboratories.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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