METHOD DEVELOPMENT AND VALIDATION OF TELMISARTAN, CHLORTHALIDONE AND METOPROLOL SUCCINATE USING RP-LC/MS EXPERIMENTAL DESIGN METHOD (CCD) IN BULK AND TABLET DOSAGE FORM

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Abstract

This research deals with optimization using the Derringer's desirability function for the development of LC-MS method for the estimation of three combination of drugs Telmisartan, Chlorthalidone and Metoprolol succinate in commercial pharmaceutical preparations used as antihypertensive. Twenty experiments, taking the retention time of the first peak, resolution between the second and third peaks and the peak area of the third peak as the responses with three important variables as acetonitrile concentration, buffer pH, and spray voltage, were used to design mathematical models. The experimental responses were fitted into a second order polynomial and the three responses were optimized to predict the optimum conditions for the effective separation of the studied compounds. The optimum conditions were acetonitrile and potassium dihydrogen ortho phosphate buffer (pH 6.8, 35:65%v/v) as the mobile phase and at a spray voltage of 2.740 V. The m/z range was found to be 481.05 → 113.25, 325.05, 585.15 → 229.10 for Telmisartan, Chlorthalidone and Metoprolol succinate respectively. The m/z range does not affect by acetonitrile concentration, buffer pH and spray voltage. The method showed a good agreement between the experimental data and predictive value throughout the studied parameter space. The optimized assay condition was validated according to the International Conference on Harmonization guidelines to confirm specificity, linearity, accuracy and precision.

Keywords: Derringer's desirability function,RP-LC/MS,Central composite design,Telmisartan, Chlorthalidone and Metoprolol succinate.

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INTRODUCTION

Telmisartan[1] chemically known as [4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2- propylben-zimidazol-1-yl] methyl] 1, 1biphenyl]-2 Carboxy-licacid, is a angiotensin receptor antagonist. Chlorthalidone[2]chemically known as 2-chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) benzene-1-sulphonamide is a diuretic. Metoprolol succinate[3] chemically known as 2-propanol, 1-[4-(2-methoxyethyl), phenoxy]-3-[(1-methylethyl) amino]- (±)-butanedioate, is a beta blocker. The estimation of telmisartan, chlorthalidone and metoprolol succinate by UV[13-15], HPLC[7-12] and LC/MS[4] methods in single and combined dosage forms. Extensive literature reveals that no methods like Method Development and Validation of Telmisartan, Chlorthalidone and Metoprolol Succinate by RP-LC/MS Experimental Design Method (CCD) in bulk and tablet Dosage Form. Quality by design is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. The central composite design could be applied to optimize the separation and to assist the development of better understanding of the interaction of several chromatographic factors on separation quality. The aim of the present work is the selection of important chromatographic factors and its optimization by a central composite design experiment.

MATERIALS AND METHODS

Chemicals and Reagents

Pure Active pharmaceutical ingredients of telmisartan, chlorthalidone and metoprolol succinate were obtained as gift samples from Nebulae Hi- Tech Laboratories, Chennai, Tamil Nādu, India. Combination tablet of Met XL 3D (telmisartan 40 mg, chlorthalidone 6.25 mg and metoprolol succinate 23.75 mg) was procured from the local market. HPLC grade Acetonitrile, HPLC grade water and analytical Potassium dihydrogen ortho phosphate were purchased from Merck Chemicals India Pvt. Limited, Mumbai, India.

Instrumentation and Chromatographic Condition

Analysis was performed with a Shimadzu LC- MS separation module equipped with Lab solution software, Pump LC 2010 binary and PDA detector set at 215 nm. Separation was conducted using Hewlett Packard 1100 series binary pumps with a phenomenax C_{18} column. A mixture of acetonitrile: potassium dihydrogen ortho phosphate (35:65) pH 6.8 was used as a mobile phase at a flowrate of 1.0ml/min. A Sciex API mass spectrometer and HPLC system were interfaced by using a turbo ion spray positive ion source with multiple reactions monitoring detection. The turbo temperature was 550°C with the spray voltage 2.740 V Nitrogen ultra-high purity and zero grade obtained from MG industries were used as the curtain gas and auxillary flow gas for the turbo (8.0 ml/min) respectively. The m/z range was found to be 481.05 → 113.25, 325.05, 585.15 → 229.10 for Telmisartan, Chlorthalidone and Metoprolol succinate respectively.

Preparation of standard stock solution

About 50 mg of Telmisartan, Chlorthalidone and Metoprolol succinate were weighed accurately and transferred into 50ml.Dissolved in acetonitrile (HPLC grade). Concentration of the solution was observed to obtain 1000 µg/ml Telmisartan, Chlorthalidone and Metoprolol succinate.

Preparation of sample solution

Estimation of Telmisartan, Chlorthalidone and Metoprolol succinate in tablet formulation by LC-MS was carried out using optimized chromatographic conditions. Twenty tablets of formulations (Met XL 3D) were weighed accurately. The average weight of the tablet was calculated and powdered. The tablet powder equivalent to 50 mg of Telmisartan, Chlorthalidone and Metoprolol succinate was weighed and transferred into 50 ml volumetric flask. About 15ml of acetonitrile was added to dissolve the substance. Then the solution was sonicated for 15mins. The volume was made up to 50 ml with the same solvent and centrifuge at 3000rpm. Then the solution was filtered through whatmann filter paper No:41 to get 30 µg/ml respectively. From the clear solution, pipetted 1.5ml of this solution was diluted to water. A steady baseline was recorded with optimized chromatographic conditions.

Chromatographic Conditions

Chromatographic separations were carried out on a C_{18} column. The mobile phase consists of acetonitrile and potassium dihydrogen ortho phosphate buffer (pH 6.8), a wavelength of 215 nm was selected. An injection volume of the sample was 20 µl.

The retention time of the first peak Telmisartan (Rt_1), the resolution of Chlorthalidone and Metoprolol succinate peak ($Rs_{2,3}$) and the peak area of the last peak Metoprolol succinate (PA_3)

were selected as responses. All experiments were conducted in randomized order to minimize the effects of uncontrolled variables that might introduce a bias on the measurements. Replicates (n=6) of the central points were performed to estimate the experimental error. For an experimental design with three factors, the model including linear, quadratic and cross terms can be expressed as

$$Y = \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_1X_1X_3+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X_3^2$$

Where Y is the response to be modeled, β is the regression coefficients and X_1, X_2 and X_3 represent factors A, B and C respectively. Statistical parameters obtained from ANOVA for the reduced models were given. The insignificant terms ($p>0.05$) were eliminated from the model through backward elimination process to obtain a simple and realistic model. Since R^2 always decreases when a regressor variable is eliminated from a regression model, in statistical modeling the adjusted R^2 which takes the number of regressor variables into account, is usually selected (Parajo J C, et al., 1992)[5].

The adjusted R^2 values were well within the acceptable limits of $R^2 \geq 0.80$ (Lundstedt T et al.,)[6], which revealed that the experimental data showed a good fit with second order polynomial equations. For all the reduced models, *p* value of < 0.05 was obtained, implying these models were significant. The adequate precision value is a measure of the signal(response)to noise(deviation) ratio. A ratio greater than 4 is desirable. The ratio was found to be in the range of 7.099 – 12.616 which indicated an adequate signal and therefore the model was significant for the separation process. The coefficient of variation (C.V) is a measure of reproducibility of the model and as a rule a model can be considered reasonably reproducible if it is less than 10%.In table 2 the interaction with the largest absolute coefficients amongthe fitted model was BC (+110.13) of PA_3model. The positive interaction between B and C was statistically significant (< 0.0001) for PA_3. The study revealed that changing the buffer pH from low to high resulted in a rapid decline in the resolution of Chlorthalidone and metoprolol succinate in the low and high levels of flow rate. Further at the buffer pH had to be at its highest level to shorten the run time.

In order to gain a better understanding of the results, the predicted models were presented in the form of perturbation plots and 3D response surface plots shown in Figure 1, 2. Variables giving quadratic and interaction terms with the largest absolute coefficients in the fitted models were chosen for the axes of the response surface plots. Perturbation plot provided silhouette views of the response surface plots where it showed how the response changes as each factor moved from a chosen reference point, with all factors held constant at the reference value. The steepest slope or curvature indicated the sensitiveness of the response to a specific factor. The factor C (spray voltage) had most important effect on phosphate buffer pH (factor B) following the acetonitrile concentration (factor A). The rest of the factors had significant effecton Rt_1 and $Rs_{2,3}$**.** Analysis of the perturbation plots and response plots of optimization Analysis of the perturbation plots and response plots of optimization models revealed that factor B and C had significant effect on the separation of the analytes. 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 present study. The identified criteria for the optimization were resolution between the peaks, resolution and peak area.

The Derringer's desirability function, D, is defined as the geometric mean, weighted or otherwise of the individual desirability functions. The expression that defines the Derringer's desirability function is:

$$D = [d_1p^2Xd_2p^2Xd_3p^2X \dots\dots\dots Xdpn]1/n$$

In criteria, the responses $Rt_1$was in the minimize in order to shorten the analysis time and $Rs_{2,3}$ was in the maximize to separate the Chlorthalidone and Metoprolol succinate. In order to separate the eluting peak Metoprolol succinate from the solvent front, $PA_3$was in the range was shown in table 3. Following the conditions and restrictions above, the optimization procedure was carried out. The response surface obtained for the global desirability function was presented. It could be concluded that there was a set of coordinates producing high desirability value (D=0.917) were Acetonitrile concentration of 31.5%, buffer pH of 6.8 and Spray voltage 2.740 and Detection at 215nm. The predicted response values corresponding to the later value of D were Rt_1= 5.176, $Rs_{2,3}$= 2.942 and PA_3= 36146.94. The observed differencebetween the predicted and experimental responses were found to be in good agreement, within a difference of 5.0% was shown in table 4. The percentage of prediction error was calculated by using the following equation

Average error = $\frac{Experimental - Predicted}{Predicted}$ x 100

TABLE 1: CENTRAL COMPOSITE DESIGN ARRANGEMENT

Run	Space Type	Factor 1 A: ACN con %v/v	Factor 2 B:pb PH	Factor 3 C:Spray voltage	Response 1 Rt_1	Response 3 $RS_{2,3}$	Response 3 Peak $area_3$
4	Center	35	6.8	2.7	5.668	2.966	35698
6	Center	35	6.8	2.7	5.668	2.966	35698
11	Center	35	6.8	2.7	5.668	2.966	35698
12	Center	35	6.8	2.7	5.668	2.966	35698
15	Center	35	6.8	2.7	5.668	2.966	35698
20	Center	35	6.8	2.7	5.668	2.966	35698
1	Axial	35	6.46364	2.7	5.668	2.966	35698
5	Axial	35	6.8	3.03636	5.598	2.914	36926
7	Axial	43.409	6.8	2.7	7.012	2.863	37061
10	Axial	26.591	6.8	2.7	4.951	2.861	37822
13	Axial	35	7.13636	2.7	5.928	2.623	37621
16	Axial	35	6.8	2.36364	5.612	2.863	34698
2	Factorial	30	7	2.9	5.296	2.725	35702
3	Factorial	30	6.6	2.9	5.121	2.796	37128
8	Factorial	30	6.6	2.5	5.094	2.727	36896
9	Factorial	40	7	2.9	6.742	2.716	36982
14	Factorial	30	7	2.5	5.162	2.749	35198
17	Factorial	40	6.6	2.9	7.301	2.865	37109
18	Factorial	40	6.6	2.5	7.295	2.855	36720
19	Factorial	40	7	2.5	6.69	2.773	35984

TABLE 2: REDUCED RESPONSE SURFACES MODELS OBTAINED FROM ANOVA

Responses	Regression model	Adjusted R^2	Model p value	% C.V	Adequate Precision
Rt_1	+5.66+0.7924*A-0.0354*B+0.0143*C-0.1759*AB-0.0129*AC+0.0191*BC+0.1710*A^2+0.1061*B^2+0.0379*C^2	0.9308	<0.0001	4.50	14.2703
$Rs_{2,3}$	2.97+0.0158*A-0.0627*B+0.0061*C-0.0227*AB-0.0115*AC-0.0200*BC-0.0506*A^2-0.0744*B^2+0.0412*C^2	0.9548	<0.0001	1.97	7.9470
Peak area 3	+35705.42+43.29*A55.13*B+429.82*C+282.63*AB+81.38*AC+110.13*BC+567.93*A^2+291.45*B^2-8.19*C^2	0.9322	<0.0001	1.99	11.8814

TABLE 3: CRITERIA FOR THE OPTIMIZATION OF THE INDIVIDUAL RESPONSES

Response	lower limit	higher limit	Criteria / Goal
Rt1	4.951	7.301	Minimize
Rs2,3	2.623	2.966	Maximize
Peakarea3	34698	37822	is in range

TABLE 4: COMPARISON OF EXPERIMENTAL AND PREDICTIVE VALUES OF DIFFERENT FUNCTIONS UNDER OPTIMAL CONDITIONS

Optimal conditions	ACN (%v/v)	Phosphate Buffer	Spray voltage	Rt_1	$Rs_{2,3}$	$Peakarea_3$ PA3
Predictive	31.508	6.728	2.740	5.176	2.942	36146.94
Experimental	31.508	6.728	2.740	5.295	2.988	36281.00
Average error				2.299	1.56	0.370
Desirability value (D) = 0.917						

TABLE 5 :VALIDATION PARAMETERS REPORTS BY LC/MS

Parameters	Telmisartan	Chlorthalidone	Metoprolol succinate

Concentration (µg/mL)	20-60	20-60	20-60
Correlation coefficient (r)	0.999	0.999	0.999
Regression equation y=mx + c	y=2979x+2225	y=255.5x+185.6	y=945.1+650.3
Slope (m)	2979	255.5	945.1
Intercept (c)	2225	185.6	650.3
LOD (µg/mL)	0.0011	0.0060	0.0207
LOQ (µg/mL)	0.0033	0.0183	0.0629

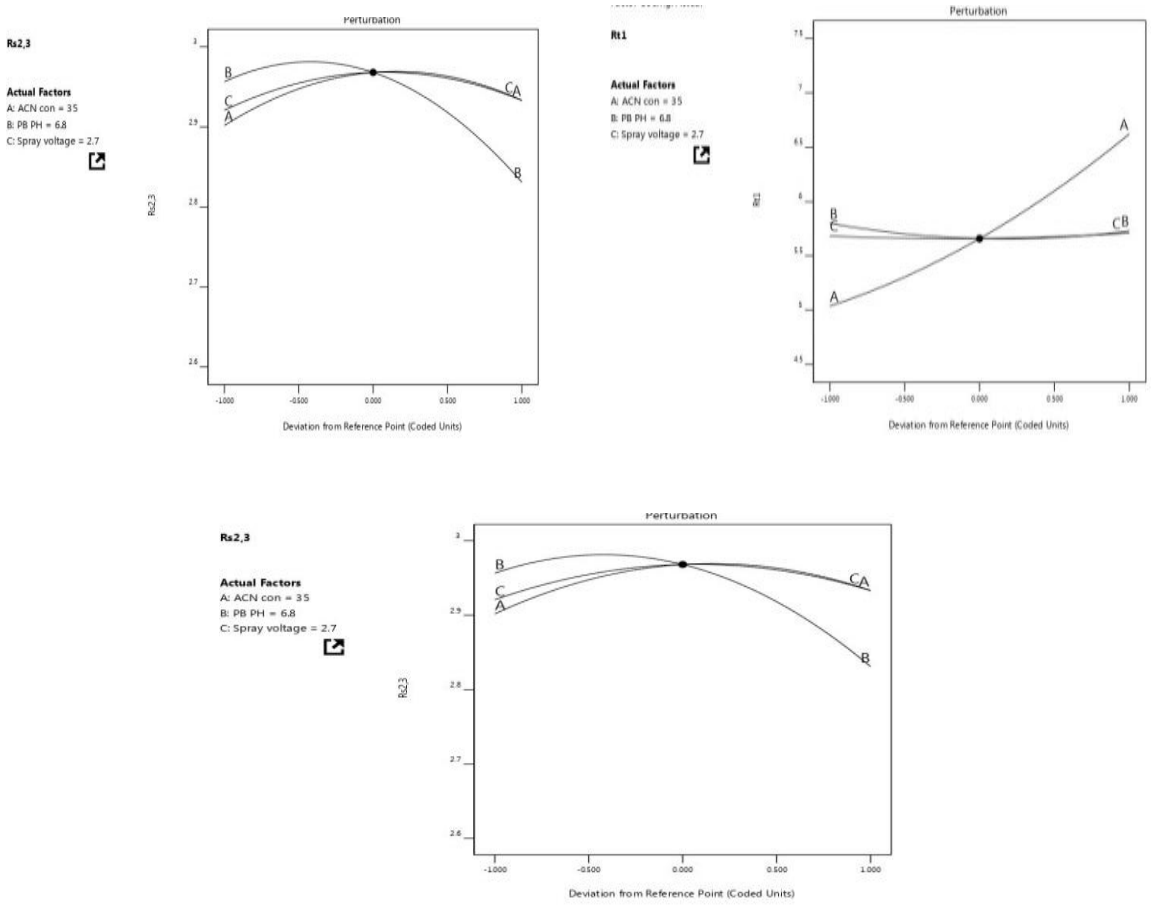
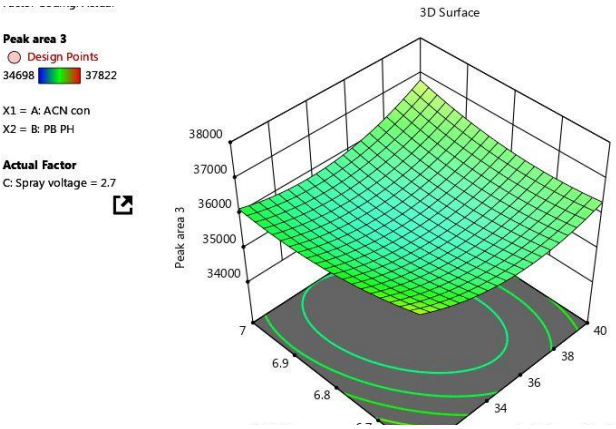
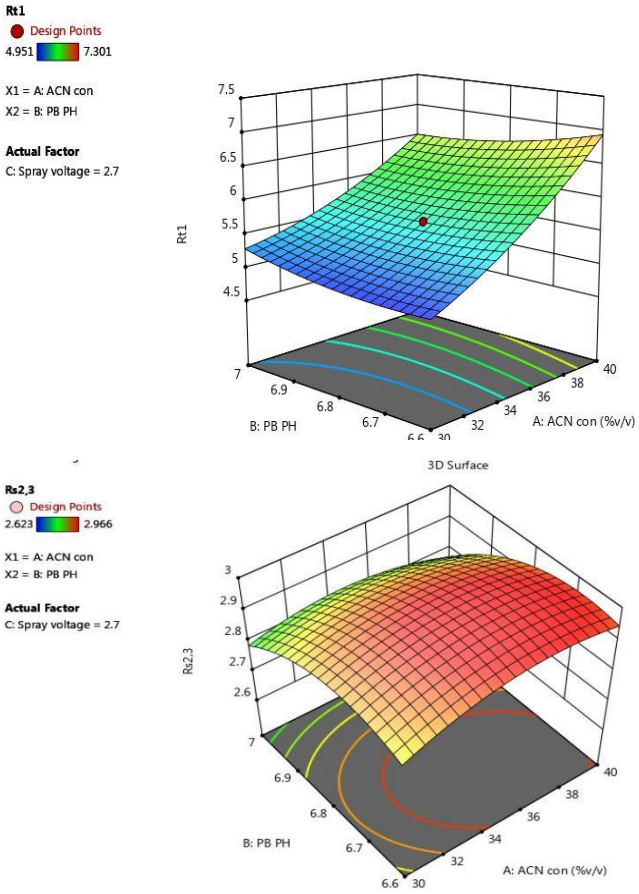


FIGURE 1: PERTURBATION PLOT FOR Rt_1, $Rs_{2,3}$ PA_3





Graphical representation for retention time, resolution and peak area – Acetonitrile (A) is plotted against Buffer (B)

FIGURE 2: RESPONSE SURFACE PLOT Rt_1, $Rs_{2,3}$ PA_3

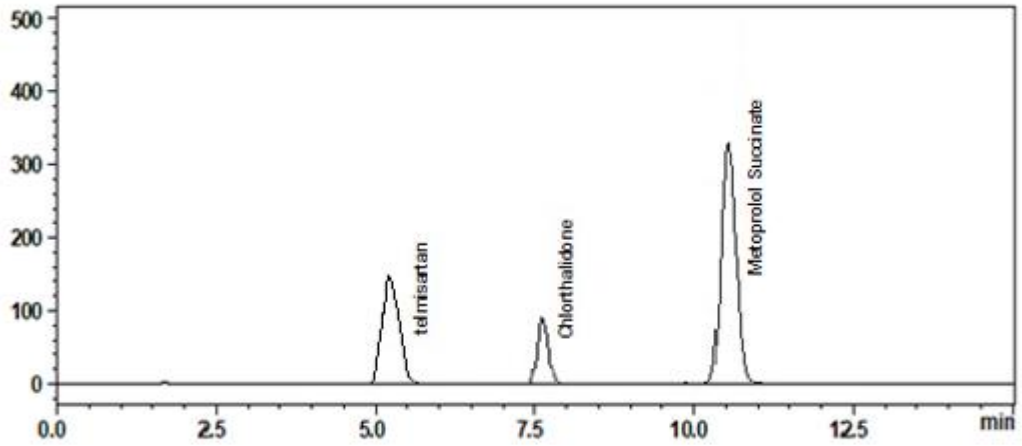


FIGURE 3: OPTIMIZED CHROMATOGRAM

METHODOLOGY

The linearity was established at five levels in the range of 20-60 µg/mL for telmisartan, chlorthalidone and metoprolol succinate. Tablet formulation MET XL 3D was selected for analysis and the percentage purity of analytes present in formulation were found to be in the range from 99.93 to 100.34%. The % RSD values were found to be 0.4021, 0.4644 and 0.1367 for Telmisartan, Chlorthalidone and Metoprolol succinate respectively.The percentage recovery of Telmisartan, Chlorthalidone and Metoprolol succinate were found to be 100.82, 99.26 and 101.01respectively. The % RSD value for Telmisartan, Chlorthalidone and Metoprolol succinate were found to be 0.5786, 0.2235 and 0.2539 %respectively. The m/z range was found to be 481.05 → 113.25, 325.05, 585.15 → 229.10 for

Telmisartan, Chlorthalidone and Metoprolol succinate.The LOD and LOQ values were in the table 5 which were found to 0.0011,0.0060,0.0207µg/mL & 0.0033,0.0183,0.0629 µg/mL for telmisartan, chlorthalidone and metoprolol succinate.

CONCLUSION

The result of the study demonstrates the benefit of applying this design in selecting optimum conditions for the drugs in pharmaceutical formulations. The m/z range do not affect by acetonitrile concentration, buffer pH, spray voltage.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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