Section A-Research paper

DEVELOPMENT OF NOVEL HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF LISINOPRIL AND AMLODIPINE IN TABLET USING QBD APPROACH

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ABSTRACT

The purpose of this research was to develop a robust, rapid, and novel high-performance thin layer chromatographic (HPTLC) method for quantitation and separation of Lisinopril and Amlodipine in combine tablet dosage form using a quality by design approach. A central composite experimental design with response surface methodology was utilized to study the effects of chromatographic chamber saturation time, band length on R_f value. The R_f value was predicted for Lisinopril and Amlodipine between 0.25 and 0.85 to optimize the chromatographic conditions based on the preliminary trials. The optimized chromatographic conditions were 15 Minute saturation time, 6 mm band length and Methanol: Toluene: Formic acid (8:2:0.2 v/v/v) as a mobile phase. The optimized HPTLC method was validated according to ICH Q2 (R1) guideline. The result of this research clearly shows that QbD approach is successfully applied to optimize HPTLC method through minimum number of experimental runs.

KEYWORDS: Central composite experimental design, optimization, quality by design, validation.

INTRODUCTION

In order to build analytical method that produce quality result with desired conditions, the quality by design (QbD) methodology is used. [1] Quality by design is substantially applicable for finding the effects of independant variable (factors) on responses by carrying out different experimental sets that are obtained from central composite design. This design is also applicable for giving maximum information about methods and factors from the minimum experimental run. At actual QbD is significant model applicable in pharmaceutical industries and is defined as per ICH regulations as "a systematic approach to the development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". [2]

From the literature, it is found that response surface method (RSM) has considerably used to optimize an analytical method, because in a response surface methodology multivariate analysis is possible, that is several factors can be optimized simultaneous [3] and the level of several factors in an experimental design domain is expected to contain optimum. The quality of (HPTLC) method is very important and is best developed by using a QbD approach, as per this approach the HPTLC method is verified at early stage of method development which give assurance about quality of method. From the literature it is clear that there are many reported papers on HPTLC method development but very few are by using a QbD environment, [4, 5] therefore in this research paper we develop HPTLC method systematically from central composite design. Amlodipine (Amlo) is calcium channel blocker, chemically it is 3-O-ethyl-5-O-methyl-2-(2-aminoethoxy methyl)-4-(2-chlorophenyl)-6- methyl-1,4-dihydro pyridine-3,5-dicarboxylate, it and used to treat heart disorders like hypertension and coronary artery diseases. Chemically Lisinopril (Lisino) is (2S)-1-[(2S)-6-amino-2-([(1S)-1-carboxy- 3-phenylpropyl] - amino) hexanoyl] pyrrolidine-2-carboxylic acid. Angiotensin converting enzyme (ACE) inhibitor and is a standard drug used to treat of critical situations of heart failure. Both these drugs are given in combination to show effects on heart disease. [6]

Literature survey revealed that there are several HPLC [7] and HPTLC [8, 9] methods for simultaneous evaluation of Lisinopril and Amlodipine in tablet and various formulations, but not a single method was developed by systematic application of QbD methodology. The rationale of this research work was to

reconnoite the importance of QbD approach in the development of HPTLC method for simultaneous evaluation of Lisinopril and Amlodipine in tablet dosage form.

EXPERIMENTAL

Instrumentation

HPTLC method development applying QbD for simultaneous evaluation of Lisinopril and Amlodipine was utilizes a Camag HPTLC system fitted with Camag thin layer chromatography (TLC) ScannerIII and sample applicator of Linomat V which is semi-automatic. The Hamilton syringe with 100 µL capacity was used to apply sample. The marketed precoated silica gel aluminum plate and flat bottom 10cm by 10 cm twin trough TLC developing chamber were used for Chromatographic separation. For the densitometric analysis TLC scanner III having Camag win CATS software was used and design expert of version 8.0 software was applied for data analysis.

Materials and Reagents

Amlopress L marketed tablet comprising 5 mg dose of Lisinopril and Amlodipine each was purchased. Methanol, Formic acid, Ethyl acetate and Toluene with analytical grade were utilized from D. Y. Patil Research centre.

Standard solution

The standard 500 µg/ml solution of Lisinopril and Amlodipine was prepared by dissolving precisely weighed 5mg of pure drug of Lisinopril and Amlodipine in a 10 ml of methanol using 10ml volumetric flask.

Application of sample

The standard solution with different concentrations and sample solution obtained from formulation were spotted on activated precoated HPTLC plates as narrow bands with 6mm band length separated by 9mm distance and dried with steam of nitrogen gas.

Optimized chromatographic specifications

Mobile phase (Methanol: Toluene: Formic acid; 8:2:0.2 v/v/v) was optimized using Central Composite response surface methodology. The 6mm of band length (optimized with Central Composite design) of sample was applied on activated HPTLC plates and were developed using above mobile phase in a specified TLC developing chamber for saturation time of 15 min at room temperature (optimized with Central Composite design). The spots on HPTLC plates were scanned using Camag TLC scanner III at absorbance mode at 338 nm.

Central composite experimental design

A Central Composite experimental design through three levels, two factors, 13 runs with four center points was selected as response surface design to evaluate quadratic, interactive, and main effects of saturation time and band length on response of R_f value of both the drug. The experimental model was analyzed for Optimization of factor levels on the response of R_f value of Lisinopril and Amlodipine.

Method validation

The optimized method obtained from experimental model was validated as per guidelines given by ICH.[17]

Linearity

The standard concentrations of 500, 1000, 1500, 2000, 2500, 3000 ng/spot of Lisinopril and Amlodipine were prepared, the peak area were determined for these concentrations using optimized method and calibration curve was obtained by plotting a graph of peak area vs.concentrations.

Precision

The peak area was determined for three different standard concentrations of 500 ng/spot, 1000 ng/spot, 1500 ng/spot of Lisinopril and Amlodipine for intraday and interday variation respectively. The method was analyzed for standard deviation, mean and relative standard deviation for obtained value of peak area.

Method Precision

Weigh 20 tablets and calculate average weight of tablet, crushed 20 tablets and weigh accurateky 245.2 mg powder and dissolve it in 10 ml of methanol. Shake the mixture for about 20 min. then filter it through whatmann filter paper, a clear solution was obtained. The 3 µl solution spot was given thrice and percent label claim was calculated from average value of peak area.

Accuracy

To determine accuracy of developed method a known amount of standard solution of 80%, 100%, and 120% were spiked in known concentration of sample solution of Lisinopril and Amlodipine and it was analyzed for three consecutive days to calculate drug recovery.

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

The formula used to calculate LOD and LOQ is LOD 3.3*SD/Slope, LOQ 10*SD/Slope. where SD is standard deviation of responses.

RESULTS AND DISCUSSION

Optimization restraint and solution

The restraints in optimization design are based on specifications of final response. The basic objective was to achieve R_f value for Lisinopril and Amlodipine in the 0.2–0.8 range. After applying a design expert software for the data obtained for the responses, the optimum condition for saturation time was 15 min and band width of 6 mm, for the mobile phase of Methanol: Toluene: Formic acid; 8:2:0.2 v/v/v.

From the preliminary data it was shown that R_f value obtained for Lisinopril was below 0.2, for the various mobile phase trials and finally Methanol: Toluene: Formic acid were found as a suitable solvent system for the above combination. As the responses of R_f value of Lisinopril and Amlodipine was found to be vary with critical factor of saturation time and band width. Lisinopril peak was observed at 0.17-0.23 R_f value for higher saturation time. The responses were also varying along with band width and optimum band width was found 6 mm. The Factors, minimum and maximum levels of factors with their units are given in Table 1 and information about responses given in Table 2.

Table 1. Chromatographic factors for central composite experimental design

Factor	Name	Units	Type	Subtype	Minimum	Maximum
A	Saturation time	Min	Numeric	Continuous	10	20
B	Band Length	Mm	Numeric	Continuous	4	8

Table2. Chromatographic response for central composite experimental design

Response	Name	Units	Obs	Analysis
Y1	R_f of Lisinopril	No unit	13	Polynomial
Y2	R_f of Amlodipine	No unit	13	Polynomial

Central composite experimental design

The random orders of experimental runs of design were accomplished to get the accurate data and results obtained are shown in Table 3. A central composite design with quadratic model was applied for finding effects of factors on R_f value of Lisinopril and Amlodipine, respectively, the equations were obtained (1) and (2) as shown below.

$$\text{Rf of Lisinopril (Y)} = +0.22+0.000*A-0.020*B-0.013*A*B-0.059*A2-0.049*B2 \quad (1)$$

$$\text{Rf of Amlodipine (Y)} = +0.82-0.020*A+0.015*B-0.035*A*B-0.11*A2-.552E-003*B2 \quad (2)$$

Where Y is the response, A and B are the factors.

Table 3. Experimental design and its responses

		Factor 1	Factor 2	Response 1	Response 2
Std	**Run**	**A:Saturation time Minute**	**B:Band Length mm**	**R_f of Lisinopril**	**R_f of Amlodipine**
13	1	15	6	0.2	0.82
7	2	15	4	0.23	0.82
3	3	10	8	0.12	0.82
8	4	15	8	0.12	0.77
5	5	10	6	0.17	0.69
12	6	15	6	0.23	0.89
10	7	15	6	0.23	0.8
9	8	15	6	0.21	0.81
1	9	10	4	0.1	0.68
2	10	20	4	0.13	0.69
11	11	15	6	0.23	0.81
4	12	20	8	0.1	0.69
6	13	20	6	0.16	0.69

Table 4 and 5 shows the ANOVA for response of R_f value of Lisinopril and Amlodipine. As per the model

probable value was found 0.0063 and 0.0351 and from this it is confirmed that model was statically significant.

Table 4. Summary of Results of ANOVA

ANOVA for central composite quadratic model for Rf of Lisinopril						
Source	**Sum of squares**	**df**	**Mean square**	**F value**	**p-value Prob > F**	
Model	0.02878	5	0.005756	8.780656	**0.0063**	Significant
A-Saturation time	-3.5E-18	1	-3.5E-18	-5.3E-15	1.0000	
B-Band Length	0.0024	1	0.0024	3.661093	0.0973	
AB	0.000625	1	0.000625	0.95341	0.3614	
A^2	0.009491	1	0.009491	14.47805	0.0067	
B^2	0.006529	1	0.006529	9.959797	0.0160	
Residual	0.004589	7	0.000656			
Lack of Fit	0.003789	3	0.001263	6.314655	0.0536	Not significant
Pure Error	0.0008	4	0.0002			
Cor Total	0.033369	12				

Table5. Summary of Results of Analysis of Variance (ANOVA)

ANOVA for central composite quadratic model for Rf of Amlodipine						
Source	**Sum of squares**	**df**	**Mean square**	**F value**	**p-value Prob > F**	
Model	0.045745	5	0.009149	4.611942	**0.0351**	Significant
A-Saturation time	0.0024	1	0.0024	1.209834	0.3077	
B-Band Length	0.00135	1	0.00135	0.680531	0.4366	
AB	0.0049	1	0.0049	2.470077	0.1600	
A^2	0.031357	1	0.031357	15.8068	0.0054	
B^2	6.65E-06	1	6.65E-06	0.003352	0.9554	
Residual	0.013886	7	0.001984			
Lack of Fit	0.008566	3	0.002855	2.146919	0.2370	Not significant
Pure Error	0.00532	4	0.00133			
Cor Total	0.059631	12				

Values for A^2B, AB^2 are not obtained due to reduced model for selected responses. Bold values given in the tables indicate that there is a significant influence of the selected factors on response. Different response surface plots for various values of factors were studied.

Figure 1 and 2 indicate the effect of factors like band length and saturation time on R_f value of Lisinopril and Amlodipine. Curve-line in plots obtained here which indicate that there is a non-linear effect of parameters of factors on R_f value.

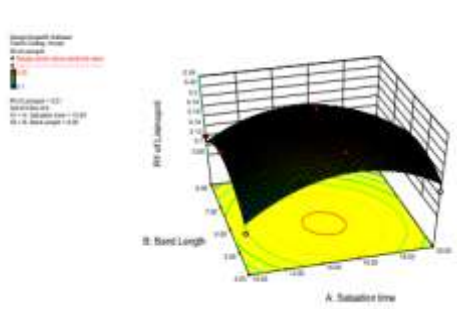

Fig. 1 Response surface plot for Lisinopril

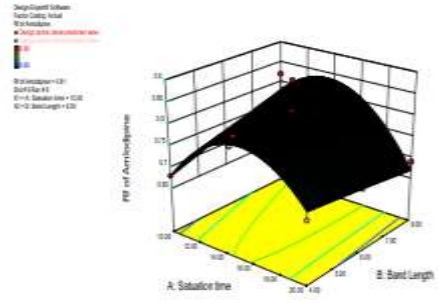

Fig.2. Response surface plot for Amlodipine

By using optimized chromatographic conditions from the central composite design the chromatographic separation of Amlodipine and Lisinopril was achieved and it shown in Figure3.

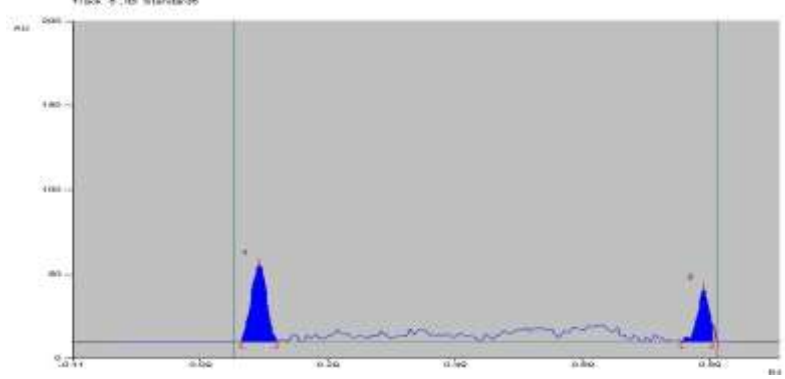

Fig. 3. Seperation of standard Lisinopril (500 ng/spot) and Amlodipine (500 ng/spot) having R_f value of 0.15 and 0.79 respectively.

METHOD VALIDATION

Linearity

The linearity curves obtained from the data was found to be linear when we plot a graph of peak area verses analyte concentration. The accepted value of correlation was obtained which is mentioned in Figure 4 and 5. The figure 6 shows a linearity curve for simultaneous evaluation of Lisinopril and Amlodipine.

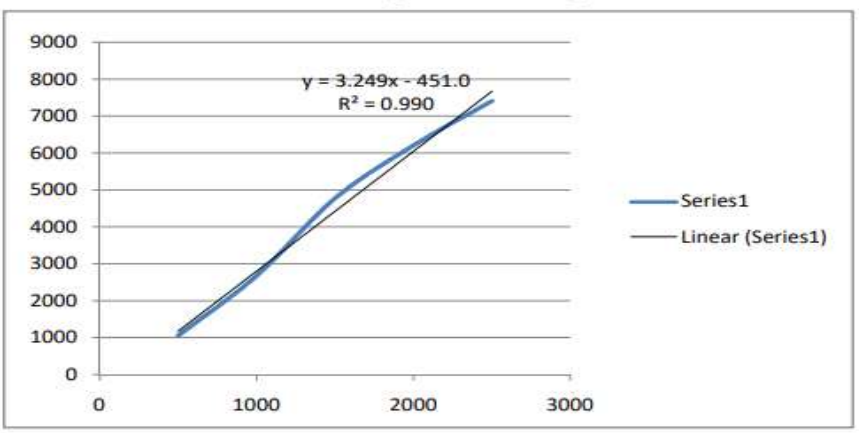

Fig. 4. Linearity curve for Lisinopril

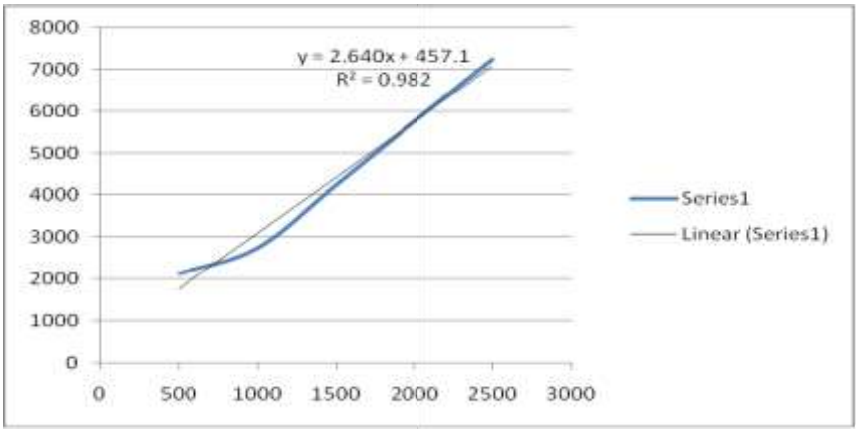

Fig. 5. Linearity curve for Amlodipine

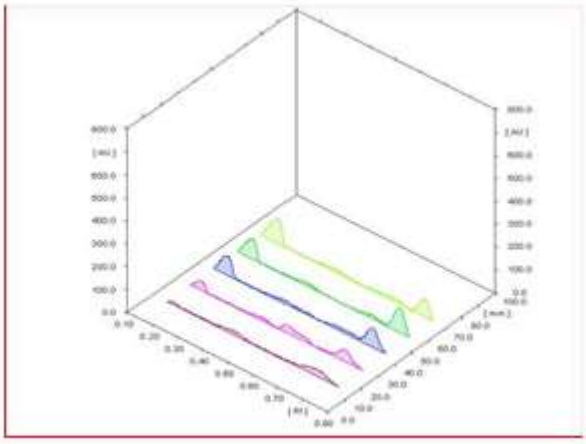

Fig. 6. Linearity for simultaneous estimation of Lisinopril and Amlodipine

Method Precision

Weigh accurately 245.2 mg of crushed powder of 20 tablet and dissolve it in 10 ml of methanol. Shake the mixture for about 20 min. then filter it through whatmann filter paper, a clear solution was obtained. The 3 µl solution spot was given thrice and percent label claim was calculated from average value of peak area. Percent label claim is mentioned in table6.

Table 7. Method Precision data

Sr. no.	**Drug content in powder**		**Percent Label Claim**	
	Amlodipine	Lisinopril	Amlodipine	Lisinopril
1.	5.09mg	5.12 mg	98.20	102.4

Precision

In case of precision we have carried out intra and inter day study, from this study data obtained were used to calculate percent relative standard deviation for the above optimized method and were found less than 2%, this concludes that the given method is precise. The data for precision study is shown in the Table 7.

Table 7. Intraday Precision Study where (n= 3)

SR. NO.	Intra Day Precision			Intra Day Precision		
	PEAK AREA OF LISINO			PEAK AREA OFAMLO		
	MORNING	AFTERNOON	EVENING	MORNING	AFTERNOON	EVENING
1	2822.4	2822.4	2726.5	3487	3261.6	3053
2	2821.8	2820.3	2762.2	3475.7	3208.2	3058
3	2821.8	2830.2	2757.3	3475.7	3250.3	3049.8
AVERAGE	2822	2824.3	2748.66	3479	3240.03	30530.7
SD	0.346	5.21	19.35	6.52	28.14	4.23
%RSD	0.012	0.18	0.7	0.17	0.86	0.138

Table 8. Inter-day Precision Study where (n= 3)

SR. NO.	Inter Day Precision			Inter Day Precision		
	PEAK AREA OF LISINO			PEAK AREA OFAMLO		
	DAY 1	DAY 2	DAY 3	DAY 1	DAY 2	DAY 3
1	3538.5	3320.6	3251	4117.6	3845.9	3470.4
2	3537.5	3340	3241	4105.2	3852.9	3485.2
3	3536.4	3314	3261	4126.3	3830	3452.3
AVERAGE	3537.46	3324.8	3251	4116.37	3842.93	3469.3
SD	1.05	13.51	10	10.6	11.73	16.47
%RSD	0.029	0.4	0.3	0.257	3842.93	0.474

Accuracy

Weigh accurately 5mg amlodipine, 5mg Lisinopril and 242.25mg of tablet powder of 20 tablets; dissolve this in 10 ml of methanol. This is a 100 percent solution, similarly the 80% and 120% solution obtained by taking 4mg, 6 mg of amlodipine and lisinopril each in 242.25 mg of powder respectively. The percent recovery was calculated and from the data given in table it is confirmed that the given method is accurate.

Table 9. Accuracy Results (n=3)

Components	Amount of standard drug added in mg	Amount estimated per tablet in mg	Amount of drug recovered in mg	Percent recovery
Amlodipine	4	9.1	4.1	102.5
	5	9.9	4.9	98
	6	10.85	5.85	97.5
Lisinopril	4	9.02	4.02	100.5
	5	10	5	100
	6	11	6	100

LOD and LOQ

Actually LOD means the lowest conc. of Lisinopril and Amlodipine which can be detected but not quantified by the method and LOQ the lowest conc. of Lisinopril and Amlodipine that can be quantified accurately and precisely by using a given method. The LOD was found 6.58 mg and 22.72 mg for Lisinopril and Amlodipine, LOQ was found 19.94 mg and 69.17 mg for Lisinopril and Amlodipine.

APPLICATION

The 20 tablets of AMLOPRESS L were weighed accurately using an analytical balance and powdered using a mortar pestle. The weight of powder was taken so that it contains 5 mg conc. of Lisinopril and Amlodipine each. By dissolving this in a methanol sample solutions were prepared, the concentration was found using a proposed method. These results shown in figure 7 indicate that method is applicable for determining concentration of drug in a marketed drug formulaton.

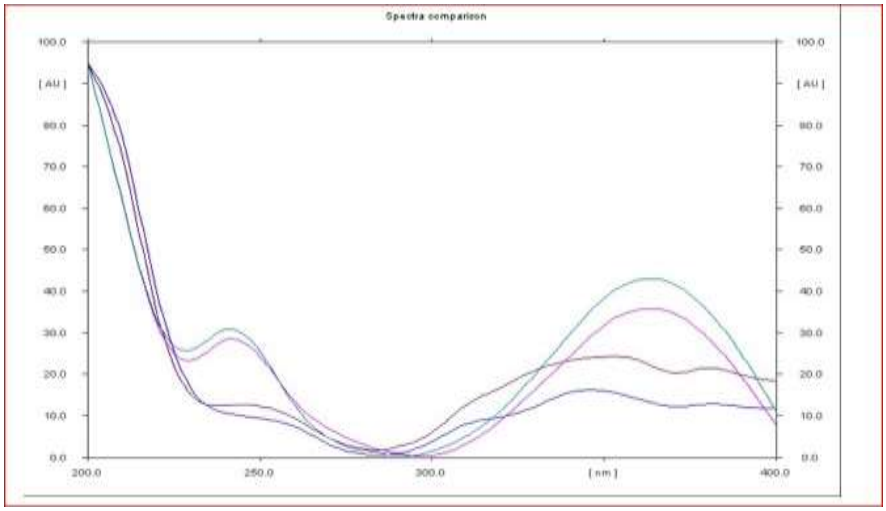

Fig. 7. Graph for standard and marketed formulation

CONCLUSION

Proficient QbD approach was used to develop a validated HPTLC method for finding a concentration of Lisinopril and Amlodipine. This approach gives better understanding of the factors which influence chromatographic separation and assure that method gives an expected result. In this study two factors were analyzed to determine their effect on response with the least number of experiments which will be possible by applying a Central composite design from design expert of version 8.0. The two factors band length and saturation time was considered in this experimental design and HPTLC method was developed. The developed method was validated for specificity, accuracy, linearity so it is concluded here that the QbD approach is successfully used to develop HPTLC method to estimate the concentration of Lisinopril and Amlodipine in a marketed tablet dosage form.

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