Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 7
Open Access

QbD based RP-HPLC Method development for Quantitative Computation of Phase III Composition Comprising Azelnidipine and Metoprolol

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Cite this paper as: Seju D. Patel, Anita Patel, Hirak V. Joshi, Ujash Shah (2024). QbD based RP-HPLC Method development for Quantitative Computation of Phase III Composition Comprising Azelnidipine and Metoprolol. *Frontiers in Health Informatics*, *13 (7) 657-668*

ABSTRACT

The research demonstrates how Quality by Design concepts and principles as prescribed by ICH guidelines can be used to formulate and evaluate Reverse Phase High Performance Liquid Chromatographic (RP- HPLC) method for Azelnidipine and Metoprolol. Factorial design approach was used with an integration of key RP-HPLC parameters of flow rate pH and percentage of acetonitrile. Optimal analysis conditions were determined through Design Expert software (Version 10.0), employing a Hypersil ODS C18 column (5.0 µ, 25 cm × 4.6 mm), Acetonitrile: Using a flow rate of 0.8 ml/min and buffer (70:30 v/v) pH 4.0, 20µg/mL. Conditions available allowed for good resolution between Metoprolol and Azelnidipine retention times of 4.55 and 14.16 respectively, and favorable system suitability parameters were obtained. At 228 nm, the linearity of the developed method was good in the ranges of 2-12 µg/ml for Azelnidipine and 12.5-75 µg/ml for Metoprolol. The optimized method was validated according to ICH guidelines on analytical method validation. Finally, it was shown to ultimately discriminate Azelnidipine from Metoprolol from the binary case, providing an efficient method for pharmaceutical analysis.

KEY WORDS

RP-HPLC, Quality by Design (QbD), Azelnidipine, Metoprolol, Analytical method validation

INTRODUCTION

Recently, Quality by Design (QbD) principles have been exploited for the development of analytical techniques due to the need for a significant level of accuracy provided by QbD guidelines defined by ICH. And particularly when reducing method variability [1,2]. This can be due to anything from different polarity solvents and buffer mixtures to being very careful about chromatographic parameters such as the composition of the mobile phase, flow rate, injection volume, pH, etc. [3]. A QbD approach first allows a scientific and risk-based understanding of the key causes of fluctuation. Then, through risk assessment and factor examination, studies attempt to identify Critical method parameters by examining factors and study. Using suitable experiment designs, these parameters are optimized [4,5]. One such vasodilator that gradually decreases the patients' blood pressure is azelnidipine (AZL). Unlike other drugs within its class, AZL does not induce vasodilation-induced reflex tachycardia. This is probably because it slowly drops blood pressure [6]. It also prevents the entry of transmembrane Ca2 into smooth muscle cell walls through voltage dependent smooth muscle channels. L type, T type, N type, P/Q type and R type Ca2+ channels exist which all differ in the way they function. L-

type 6 Ca2+ channels [7]. If you normally contract smooth muscle with calcium, then it will lead to hypertension. Calcium channels stopped working, which means vascular smooth muscle relaxes and blood pressure comes down because the vascular smooth muscles don't contract [8]. A beta-1 selective blocker, metoprolol (MET), is available in tartrate and succinate derivatives and is manufactured for extended or immediate-release formulations. The limited systemic bioavailability of its succinate derivative enables the creation of these formulations [9]. Inhibition of beta-1-adrenergic receptor (beta-1-adrenoceptor) in cardiac cells with specific action on beta-2 receptor, but not on the inherent sympathomimetic and membrane stabilizing activity, gives negative chronotropic and chronotropic activity that decreases blood flow without intrinsic sympathomimetic effects [5]. A beta-1-adrenergic receptor is one of the MET inhibitors [10]. This combination of MET succinate, a beta blocker, and AZL, a new dihydropyridine calcium channel antagonist, was approved by CDSCO for Phase III clinical investigation in April 2021. This combination is used in stage 2 hypertension when it is treated [11]. All the data generated and analyzed for this study paper was included [12].

MATERIALS AND METHODS

Instruments and chemicals

The samples were processed using the SHIMADZU (Series 2010) HPLC apparatus equipped with a UV-VIS detector with binary gradient operation capability. LC solution software was used to process and integrate data acquired in chromatographic analysis. In the process a HYPERSIL ODS C18 column (250 mm x 4.6 mm i.d.) was employed. Material quantities were determined using a Mettler Toledo weighing scale with a sensitivity of 0.1 mg and pH adjustments with the Labman LMPH 10 pH meter. Complimentary samples of the active pharmaceutical ingredients were supplied by Alembic Pharmaceutical Pvt. Ltd., Vadodara, Gujarat, India and Sunij Pharma Pvt. Ltd., Vatva, Ahmedabad, Gujarat, India. Various chemicals such as O-phosphoric acid, water, Acetonitrile, KH2PO4 buffer were purchased from Merck Life Sciences Private Limited (Mumbai, India) all meeting standards of HPLC quality.

Factor screening studies

Following an initial literature review, specific critical method parameters (CMPs) such as flow rate, proportion of the Organic phase, and pH were selected for exploration of factors. Finally, these parameters were transformed into a matrix to explore how each works collectively to impact critical method attributes (CMAs), such as retention duration, resolution, and peak tailing [13-15]. The framework detailing critical method parameters and their assigned levels that impact the CMAs is shown in table no.1.

Preparation of Standard Solution

To prepare the standard solution, accurately weighed amount of AZL (8 mg) and MET (50 mg) was taken into the volumetric flask (10 ml) and volume of the flask was raised to 10 ml with acetonitrile to give stock solutions containing 800 µg/ml of AZL and 5000 µg/ml of MET. From this solution, take out 10 ml & makeup to 100 ml with the mobile phase & finally, from this solution, take 1 ml & volume adjust with 10 ml with the mobile phase to give the final solution containing 8+50 µg/ml of AZL and MET [16].

Experimental design for Method development

To determine the analytical wavelength for method development, a solution containing 8 µg/ml of AZL and 50 µg/mL of MET underwent individual scanning between 200-400 nm. It's not only the identification of Critical Method Parameters (CMPs) that holds importance; rather, it's the combined effect of all CMPs observed during chromatographic separation. A three-factorial design was employed to investigate the combined impact of each CMP on the aforementioned Critical Method Attributes (CMAs). Design Expert 10.0 was utilized to formulate the three-factorial design for the study, generating a 27 trial runs, as detailed in Table 2. All experimental runs assessing CMAs (retention duration, resolution, and peak tailing) utilized a standard concentration of 8 microgram per ml of AZL and 50 microgram per ml of MET [17].

Optimizing And analyzing data

Using Design Expert, data was analyzed and optimized using multiple linear regression analysis (MLRA) and a quadratic

design model. The polynomial equation was built using model coefficients with a statistically significant P Value of 0.05. Finally, a variety of metrics, including lack of fit analysis, projected error sum of squares, and coefficient of correlation, were used to assess the model's applicability for application. Response surface analysis was performed on the 2D-contour and 3D-response surface plots in order to identify the factor-response relationship and any interaction effect(s). The ideal chromatographic condition was achieved by maximizing both attractiveness and numerical functions [18].

Simulation of desired conditions to chromatographic output

The Design Expert software's desired data was used to replicate the circumstances and perform a chromatography on a solution containing 8 µg/mL of AZL and 50 µg/mL of MET. After obtaining the chromatogram, the system suitability parameters were verified three times, and the mean real values were compared with the expected values [19].

Validation of Optimized method

The enhanced methodology was verified adhering to the ICH Q2R2 guideline. By injecting five different doses of AZL (ranging from 2-12 µg/ml) and MET (ranging from 12.5-75 µg/ml), a standard calibration curve was constructed for the linearity. There was a linear calibration curve between peak area and drug concentration. To verify the linearity, linear regression analysis was performed. The method's repeatability was confirmed by injecting a 100% concentration of AZL (8 µg/mL) and MET (50 µg/mL) six times in total, while keeping an eye on the relative standard deviation (RSD). We used statistical methods to determine LOD and LOQ. The mixture including the whole range was analyzed three times for intraday precision, and the same concentration (AZL+MET = 2+2.5, 8+50, and 12+75 µg/mL) was also analysed on different days for interday precision monitoring. To assess the accuracy of the approach, RSD was tracked [20]. The method's accuracy was evaluated by tampering a placebo with standard. The desired concentration was 8+50 µg/mL for AZL + MET. At 50, 100, and 150% of the targeted concentration, the placebo was spiked. For the aforementioned category, which includes Directly Compressible Lactose (100 mg), magnesium stearate (2 mg) and talc (2 mg), the placebo composition was chosen based on its broad use. Lactose served as an immediately compressible material. Talc serves as a lubricant, and magnesium stearate was utilized as a gliding agent. The percentage recovery was tracked while three replicates at each concentration were examined [21].

Quantification of AZL and MET from Synthetic Mixture

The previously stated 104 mg placebo, 8 mg AZL, and 50 mg MET made up the proposed active binary mixture. The components were diluted in 10 milliliters of acetonitrile to yield a mixture that contained, respectively, 800 µg/ml of AZL and 5000 µg/ml of MET. A Whatman filter disk measuring 0.45 m was used to filter the solution previously reported. The filtrate was diluted to 10 mL with mobile phase, yielding a solution containing 8 µg/ml of AZL and 50 µg/ml of MET .To find the percentage assay, the mixture was tested in triplicate [22].

RESULTS AND DISCUSSION

Effect of Independent variables on responses

Dose and absorptivity were varied, and the only constant was the analytical wavelength, which was chosen after much consideration based on large variations in dose. As the chromatoogram's AZL and MET signals were sufficient at 228 nm (Figure 2), this analytical wavelength was chosen.

The noted reactions following the route implementation of the factorial design methodology are presented in Table 3. We made the following findings after statistically processing the replies in Design Expert software. The model's performance was tested by comparing Adj R^2 with Pred R^2; for all responses, the difference between the two should be less than 0.2. Another phrase noted in the history of Adeq Precision is that all responses should have a ratio greater than 4. We identified Response 1 as having model terms A, B, C, and A2 that are important for modeling. The Adeq Precision of 10.513 indicated a high signal. The presented design was used to explore the design space indicated as the Surface and counterplot (Figure 3). The polynomial equation represented MET = +4.20 + 0.090* A - 0.13*B - 0.26*C - 0.21*A2 (Reduced model, as AB, AC, BC, B2, and C2 are nonsignificant terms). It was shown that A, B, C, and B2

were significant model terms for response 2 (Rt of AZL). The Adeq Precision value of 32.717 indicated a sufficient signal. The surface and counterplot of this same model are stressed in Figure 4. The polynomial problem model act as a reduced model of RT AZL = Rt of AZL = +13.67 -0.096 * A -0.25 * B -0.38 * C -0.12 * B2. Response 3 (Tf of MET) was found to have significant model terms A, B, and C. The Adeq Precision value of 25.189 proved to be a sufficient signal. In Figure 5, a highlighted surface and counterplot of the same model are shown. The form of the reduced equation of the polynomial equation that the TF MET = +1.37 + 0.032 * A + 0.064 * B + 0.033 * C is significant, and the TF of AZL was significant response 4 (Tf of AZL). The Adeq precision value of 22.164 was a sufficient signal. The surface and counterplot of the same model in Figure 6 are emphasized. The assumed reduction model would be TF AZL = +1.25 +0.091A +0.10B +0.094C -0.063AB. Model terms A and B were essential for Response 5 and terms A, B, and C were essential for Response 5. The Adeq Precision value of 24.943 indicated a sufficient signal—figure 7 concentrates on emphasizing surface and counterplots of the same model. The value for the resolution to the polynomial equation is just +13.79 -0.34 * A -0.49 * B -0.41 * C. Based on the aforementioned data, a desirability surface plot (Figure 8) was created which indicated the generality of requested conditions under which the best response is expected for the dependent variables. We anticipated 72.7 volumes of ACN, 0.8 millilitres per minute of flow, and a pH of 3.5. The data on this sheet showed that 0.683 indicated desire for the given condition — this tells us something about the concept of perfect fit. Under the conditions suggested, the design expert program was anticipated to give the optimal response. Subjects replicating the software obtained circumstances showed perfect separation with the conditions (Figure 9). Table 4 shows that the precited values are exactly matched with the real values in the responses. Under these circumstances, all of the system suitability metrics fall within the ranges shown in Table 5.

Method Validation

The developed method had linear correlation R2 values for AZL (2–12 µg/ml) and MET (12.5-75 µg/ml) were 0.9992 and 0.9992, respectively. concentration ranges (Figure 10). With the percentage recovery falling between 98 and102, this showed that all of the responses fell within the necessary acceptable range and that there was a good degree of resemblance between the observed and predicted data for the accuracy studies at the 50, 100, and 150% levels. After performing testing for repeatability and Intermediate Precision the percentage RSD values were found to be less than 2%. The technique validation parameters are outlined in Table 6.

Quantification from Binary Mixture

The amount found for AZL was 7.79 + 0.07 µg/ml (99.63 + 0.82 %w/w), and for MET, it was 49.64 + 0.32 µg/ml (99.29 + 0.64 %w/w) when the newly developed and validated procedure was applied to a mixture containing 8 µg/ml of AZL and 50 µg/ml of MET. No interference from the placebo components was observed.

CONCLUSION

A reliable and strong RP-HPLC method for AZL and MET was successfully built with a desirability of 0.842, indicating the best achievable separation, using Design Expert 10 software. Acetonitrile: It was also mentioned that under 20µg/mL Buffer (70:30 v/v), pH 4.0 and 0.8 ml/min flow rate. When run in the expected circumstances every response was exactly in line with the expected values. Finally, the application of this technique was validated for AZL and MET measurement of the binary combination with assayed percentage of 99.63 and 99.29%, respectively, following ICH Q2R2 compliance. The QbD technique was found, according to the outcome of risk assessment and method development experience, to show remarkable linearity, accuracy, precision, Since this new combination lacks any HPLC technique to determine, the determination is robust.

AUTHORS' CONTRIBUTIONS

The research was carried out under the direction of Ms. Seju Patel, who was in charge of idea generation, design, data collection, analysis, and interpretation; Dr. Anita Patel was in charge of statistical data analysis and text revision; Dr. Ujash Shah was in charge of funding and material acquisition; and Dr. Anita Patel assisted with managing the entire research process and correspondence.

Acknowledgements

The authors express their gratitude to RMS Scientific Services, Anand, Gujarat, for providing a free sample of Azelnidipine for this study, and Metoprolol. The management of A- One Pharmacy College provided excellent research resources, for which the authors are grateful.

FINANCIAL SUPPORT

Funding has not been received or reported for the work that has been done.

CONFLICT OF INTEREST

The writers have disclosed no financial or other conflicts of interest.

DATA AVAILABILITY

This study paper includes all generated and analyzed data.

ETHICAL APPROVALS

There are no experimental animals, animal components, or human beings used in this study project.

List of tables

Table 1. Factorial design variables

Factors	**Factors level**		
	-1	**0**	**+1**
Amount of ACN (X1)	60%	70%	80%
pH (X2)	3.5	4	4.5
Flow rate (X3)	0.8	1	1.2

Table 2. Executing three factorial design (coded values)

	Actual Value			**Coded Value**		
Run	**Factor 1 Proportion of Acetonitrile**	**Factor 2 Flow rate**	**Factor 3 pH of mobile phase**	**Factor 1**	**Factor 2**	**Factor 3**
1	80	1.2	3.5	1	1	-1
2	80	1.2	4	1	1	0
3	80	1.2	4.5	1	1	1
4	80	1	3.5	1	0	-1
5	80	1	4	1	0	0
6	80	1	4.5	1	0	1
7	80	0.8	3.5	1	-1	-1
8	80	0.8	4	1	-1	0
9	80	0.8	4.5	1	-1	1
10	70	1.2	3.5	0	1	-1
11	70	1.2	4	0	1	0
12	70	1.2	4.5	0	1	1
13	70	1	3.5	0	0	-1
14	70	1	4	0	0	0
15	70	1	4.5	0	0	1
16	70	0.8	3.5	0	-1	-1
17	70	0.8	4	0	-1	0
18	70	0.8	4.5	0	-1	1
19	60	1.2	3.5	-1	1	-1

20	60	1.2	4	-1	1	0
21	60	1.2	4.5	-1	1	1
22	60	1	3.5	-1	0	-1
23	60	1	4	-1	0	0
24	60	1	4.5	-1	0	1
25	60	0.8	3.5	-1	-1	-1
26	60	0.8	4	-1	-1	0
27	60	0.8	4.5	-1	-1	1

Table 3. Matrixes of factorial experimental design with responses

Run	Factor A Proportion of Organic Phase	Factor B Flow rate	Factor C pH of mobile phase	R1 (R_t of MET)	R2 (R_t of AZL)	R3 (T_f of MET)	R4 (T_f of AZL)	R5 (Resolution)
1	80	1.2	3.5	4.018	13.503	1.437	1.283	12.901
2	80	1.2	4	3.826	13.122	1.493	1.391	12.566
3	80	1.2	4.5	3.518	12.718	1.512	1.487	12.102
4	80	1	3.5	4.309	13.902	1.342	1.243	13.921
5	80	1	4	4.016	13.516	1.391	1.286	13.653
6	80	1	4.5	4.002	13.017	1.408	1.431	13.310
7	80	0.8	3.5	4.387	13.943	1.321	1.222	14.512
8	80	0.8	4	4.154	13.684	1.386	1.269	14.098
9	80	0.8	4.5	3.921	13.258	1.401	1.317	13.612
10	70	1.2	3.5	4.285	13.706	1.412	1.207	13.973
11	70	1.2	4	3.981	13.432	1.471	1.371	13.681
12	70	1.2	4.5	3.764	12.918	1.494	1.463	13.412
13	70	1	3.5	4.079	13.991	1.306	1.181	14.216
14	70	1	4	4.188	13.642	1.321	1.214	13.987
15	70	1	4.5	4.107	13.246	1.384	1.426	13.544
16	70	0.8	3.5	4.516	14.181	1.241	1.124	14.634
17	70	0.8	4	4.207	13.708	1.293	1.193	14.129
18	70	0.8	4.5	4.112	13.266	1.326	1.308	13.703
19	60	1.2	3.5	4.102	13.614	1.371	1.184	14.111
20	60	1.2	4	3.714	13.317	1.392	1.352	13.726
21	60	1.2	4.5	3.414	12.808	1.408	1.402	13.514
22	60	1	3.5	4.124	14.014	1.323	1.146	14.614
23	60	1	4	3.913	13.716	1.354	1.162	14.208
24	60	1	4.5	3.564	13.402	1.381	1.209	13.686
25	60	0.8	3.5	4.407	14.201	1.276	0.817	14.913
26	60	0.8	4	4.199	13.814	1.294	0.953	14.555
27	60	0.8	4.5	3.087	13.513	1.308	1.058	13.871

Table 4 Comparison between Predicted and actual values of Dependent variables

Factor	Predicted Value	Actual Value
Retention time of MET (R1)	4.53	4.55
Retention time of AZL (R2)	14.10	14.16
Tailing Factor of MET (R3)	1.28	1.29
Tailing Factor of AZL (R4)	1.10	1.11
Resolution (R5)	14.59	14.42
Desirability	0.683	

Table 5 System Suitability Parameters under optimized chromatographic conditions

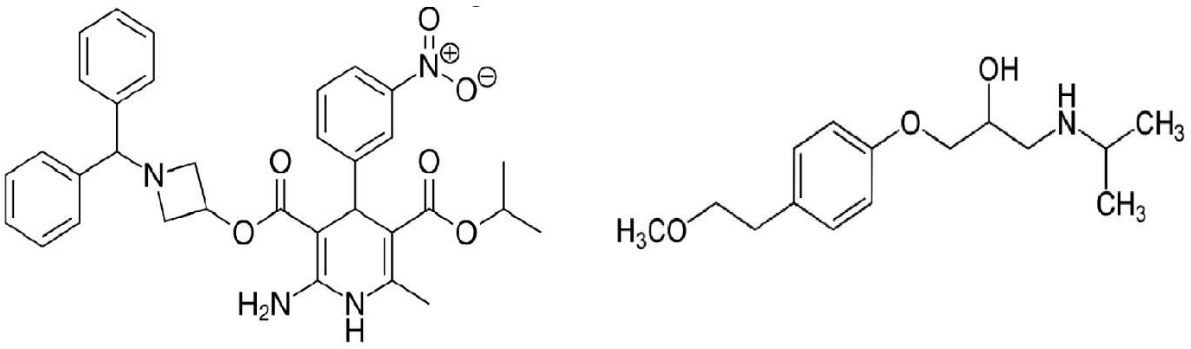
Parameter	MET	AZL
Retention time (R_t) [min.]	14.12 ± 0.01	4.54 ± 0.01
Tailing Factor	1.11 ± 0.01	1.28 ± 0.01
Number of theoretical plates [plates/meter]	6936.33 ± 17.62	1155.33 ± 57.19
Resolution [R_s]	14.46 ± 0.11	

(n=5 determinations)

Table 6. HPLC Validation Summary for Quantification of Metoprolol and Azelnidipine

Parameter	Limit	Result		Inference
		MET	AZL	
Linearity and Range	R^2 >0.999	0.9992	0.9992	Method is Linear
Repeatability	%RSD < 2	0.79	0.87	Method is Repeatable
Inter-day precision	%RSD < 2	0.77-1.10	0.72-1.13	Method is Precise
Intraday precision	%RSD < 2	1.11-1.47	0.84-1.22	Method is precise
% Recovery	98-102%	99.39-99.41	98.78-100.33	Method is Accurate
Assay	-	99.29	99.63	-

List of Figures



(A) (B)

Figure 1 Chemical structures of A. Azelnidipine and B. Metoprolol

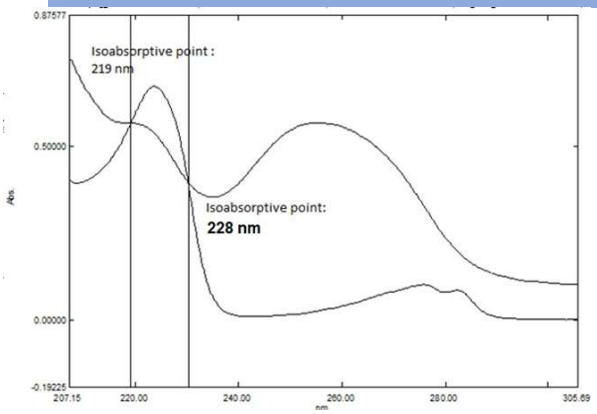
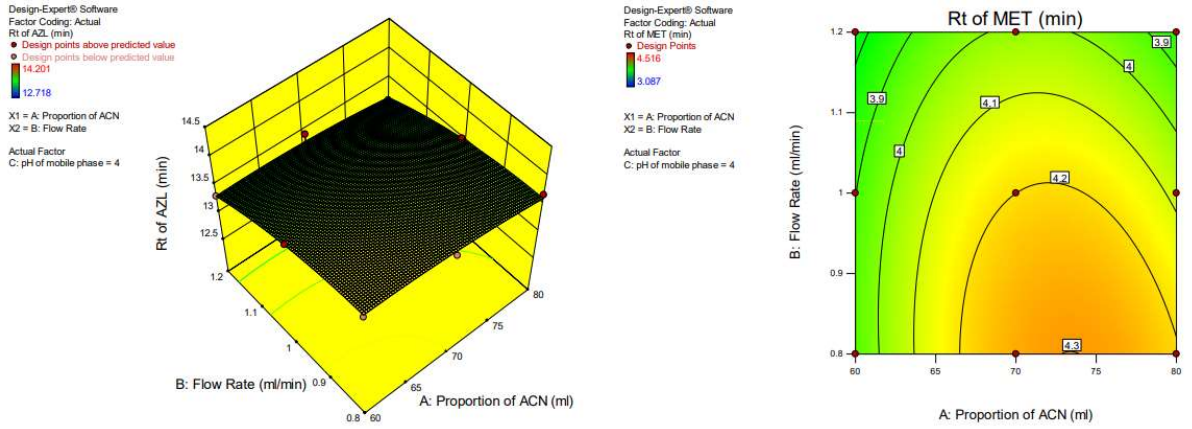
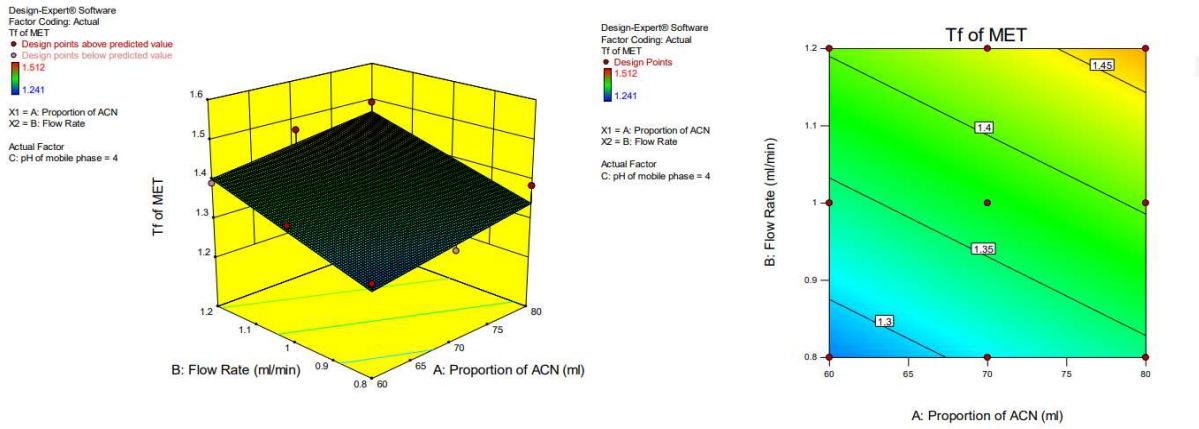


Figure 2 Overlain UV spectrum of AZL (8 µg/ml) and MET (50 µg/ml)



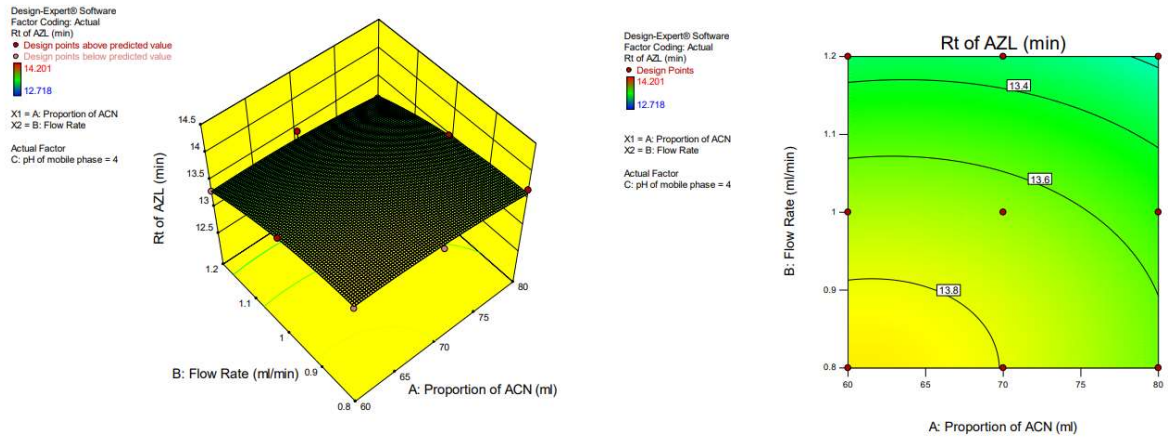
3D representation of effect of independent variables on R1 Counter plot of response 1 (Rt of MET)

Figure 3 Effect of Independent variable on Retention of MET



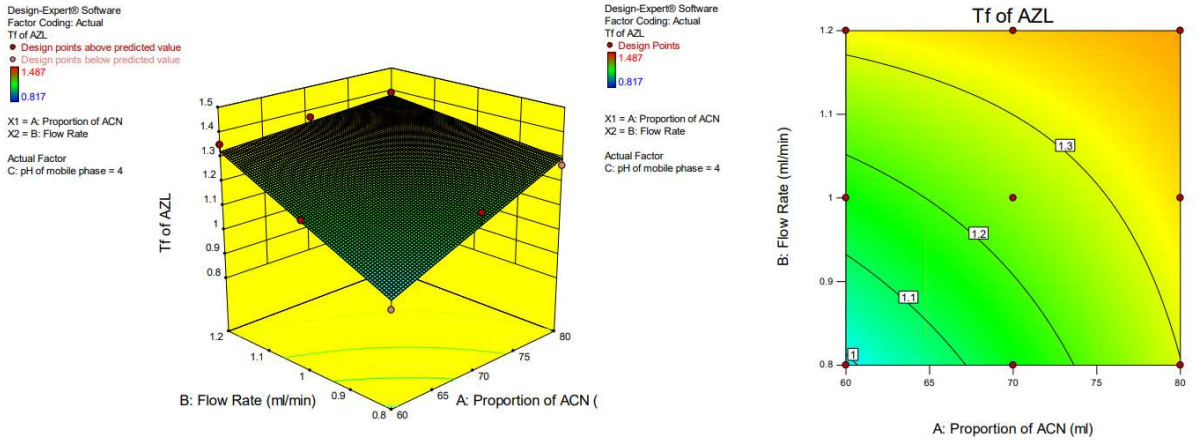
3D representation of effect of independent variables on R3 Counter plot of response 3 (Tailing factor of MET)

Figure 4 Effect of Independent variable on Tailing factor of MET



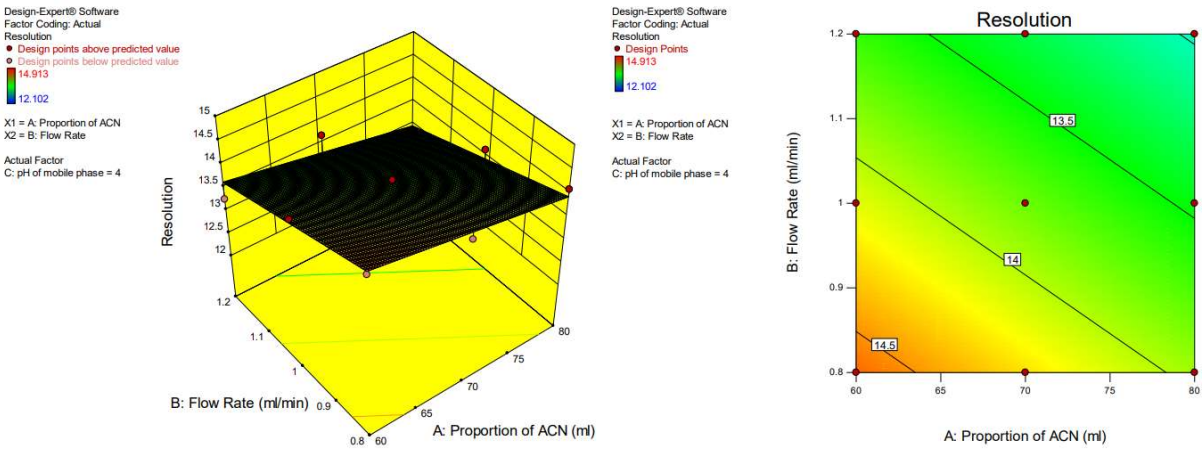
3D representation of effect of independent variables on R2 Counter plot for response 2

Figure 5 Effect of Independent variable on Retention of AZL



3D representation of effect of independent variables on R4 Counter plot for response 4 (Tailing factor of AZL)

Figure 6 Effect of Independent variable on Tailing factor of AZL



3D representation of effect of independent variables on R5 Counter plot for response 5

Figure 7 Effect of Independent variable on Resolution

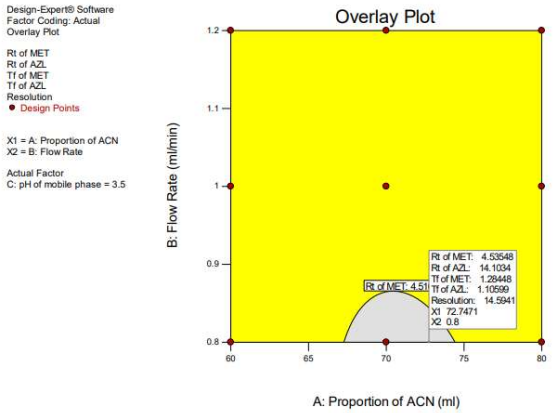


Figure 8 Desirability Plot

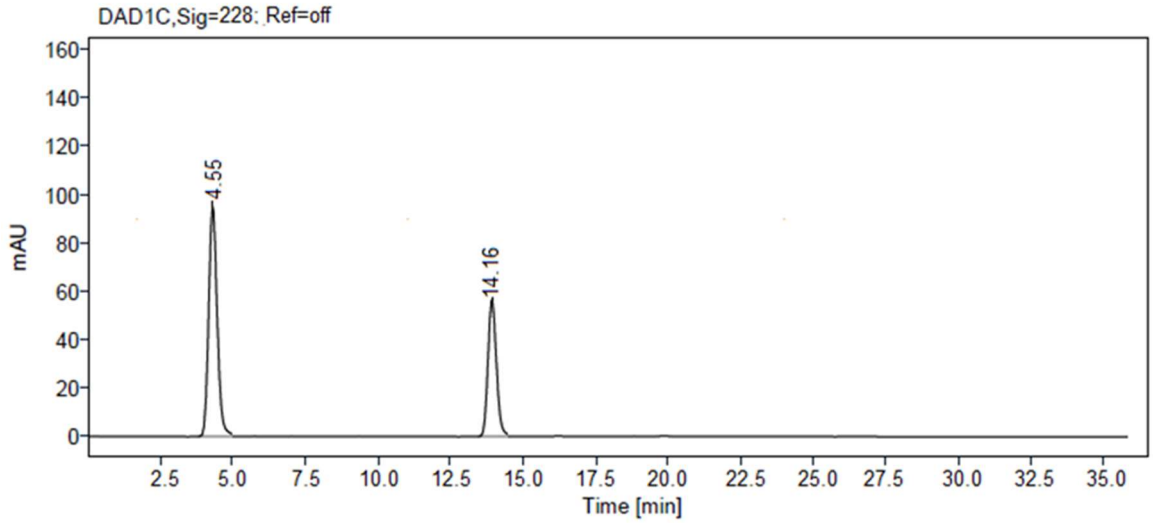


Figure 9 Chromatogram under Optimized Conditions

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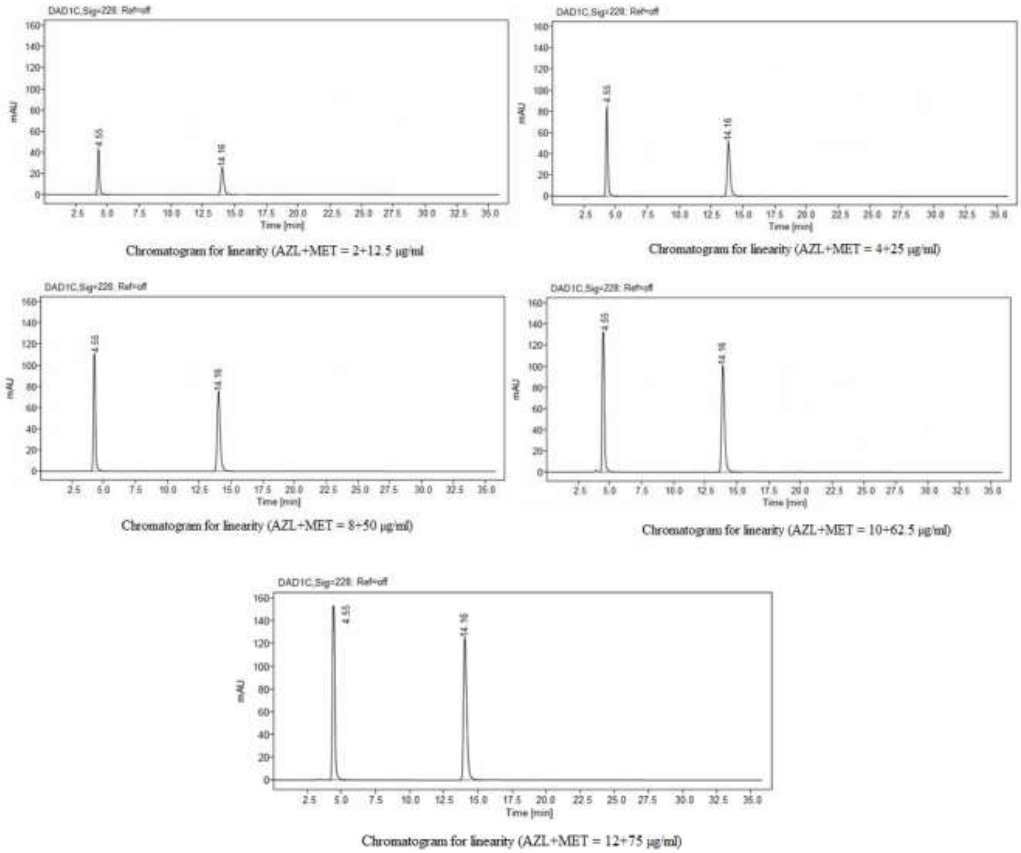

Figure 10 Chromatogram for linearity

REFERENCES

1. Monks K, Molnar I, Rieger HJ, Bogati B, Szabo E, Quality by design: multidimensional exploration of the design space in high performance liquid chromatography method development for better robustness before validation, Journal of Chromatography A, 2011; 1232: 218–30.

2. Orlandini S, Pinzauti S, Furlanetto S, Application of quality by design to the development of analytical separation methods, Analytical Bioanalytical Chemistry, 2013; 405: 443–50.

3. Mehta B, Joshi H. Shah U, Patel P, Implementation of QbD Principles for Simultaneous Quantitative Expression of Olmesartan Medoxomil, Telmisartan and Hydrochlorothiazide by RP-HPLC, Journal of Pharmaceutical Research International, 2021; 33(41A): 50–61.

4. Patel S, Ismail Y, Singh S, Rathi S, Shakya S, Patil SS, Bumrela S, Jain PC, Goswami P, Singh S. Recent Innovations and Future Perspectives in Transferosomes for Transdermal Drug Delivery in Therapeutic and Pharmacological Applications. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2024 Oct 24;40:e20240031. doi: 10.62958/j.cjap.2024.031. PMID: 39442957.

5. Horyn M, Logoyda L, Methods of metoprolol analysis in drugs and biological fluids: Review and suggestions, International Journal of Medicine and Medical Research, 2019; 5(2): 88-97.

6. Hitanshi D, Zarana D, Simultaneous estimation of Azelnidipine and Metoprolol succinate with greenness assessment using HPLC and UV-spectrophotometric methods, Green Analytical Chemistry, 2023; 7:100079.

7. Kunti S, Mrunali P, UV-spectrophotometric method development and validation for determination of Azelnidipine in pharmaceutical dosage form, International Journal of Pharmacy and Pharmaceutical Sciences, 2011; 4(1):238-240.

8. Manisha P, Vasudha D, Srinivas R, Vara P, RP-HPLC method for determination of Azelnidipine and Telmisartan in pharmaceutical dosage form, International Journal of Pharmacy and Pharmaceutical Sciences, 2023, 4(1):238-240
9. Sneha U, Kalshetty M, Bhavana H, Jyoti M, Sagar A, Development and validation of RP-HPLC for quantification of Azelnidipine in tablet, International journal of creative research thoughts, 2021, 9(7):1-6.
10. Jian-jun Z, Hong-Jian Z, Xiao-Hua Z, Yu-Bin Z, Hong-Wei F, Da-Wei X, Qin H, Determination of azelnidipine by LC-ESI-MS and its application to a pharmacokinetic study in healthy Chinese volunteers, Die Pharmazie-An International Journal of Pharmaceutical Sciences, 2008, 63(8):568-70.
11. Jain P, Patel M, Bari S, Surana S, Development and Validation of HPTLC Method for Simultaneous Determination of Amlodipine Besylate and Metoprolol Succinate in Bulk and Tablets, Indian Journal of Pharmaceutical Sciences, 2012, 74(2): 152–156.
12. Abhinaya K, Shyamala D, Irfana S, Pawan M, Sahithi G, Saikrishna A Chinnaeswaraiah A, UV spectrophotometric method development and validation for the determination of metoprolol succinate in bulk and its pharmaceutical dosage form, Annals of Phytomedicine, 2023, 12(1): 628-631.
13. Bindi V, Rajesh P, Pratik N, Dushyant S, Development and validation of the simultaneous UV spectrophotometric method for estimation of metoprolol succinate and olmesartan medoxomil in the tablet dosage form, Pharmaceutical Methods, 2012, 3(1): 44–47.
14. Rawool N, Venkatchalam A, Analytical Method for the Simultaneous Estimation of Hydrochlorothiazide and Metoprolol Tartrate using RP HPLC, Indian Journal of Pharmaceutical Science, 2011, 73(2): 219–223.
15. Mahvash I, Shobha R, Estimation of Metoprolol in human plasma by HPLC method, International Journal of Pharmacy and Pharmaceutical Sciences, 2014, 7:(1), 442-446.
16. Kalshetti M, Kankure S, Development and validation of RP-HPLC method for simultaneous estimation of metoprolol, telmisartan and clinidipine in tablet, International Journal of Pharmaceutical Sciences and Research, 2020, 37, 1651-1657.
17. Mukkanti M, Lakshmana A, Development and validation of a LC-MS/MS method for simultaneous quantification of Ivabradine and Metoprolol in rat plasma, Journal of Pharmacological and Toxicological Methods, 2022, 116, 107186.
18. Rizk M, Shereen M, Mariam M ,Maha M, Comparative HPTLC study for simultaneous determination of ivabradine and metoprolol using UV and fluorescence detectors, BMC Chemistry, 2023, 17(1): 113.
19. Sathe S, Sanjay B, Sanjay S, Development of HPTLC method for the estimation of Metoprolol succinate in bulk and in tablet dosage form, Indian Journal of Pharmaceutical Education and Research, 2008, 42(1):32-35.
20. Kamini S, Janhavi R, Raja R, Kev N, Validated HPTLC method for simultaneous estimation of metoprolol succinate and ramipril in bulk drug and marketed formulation, Clinical and Medical Reports, 2019, 2: 2-4.
21. Anamika S, Aarti R, Goshiya K, Garima C, An RP-HPLC Method Performance and Validation for Azelnidipine Measurement and Metoprolol Succinate Within a Synthetic Mixture, Pharmacophore, 2023, 14(3):1-6.
22. Singh S, Chaurasia A, Gupta N, Rajput DS. Effect of Formulation Parameters on Enalapril Maleate Mucoadhesive Buccal Tablet Using Quality by Design (QbD) Approach. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2024 Jun 27;40:e20240003. doi: 10.62958/j.cjap.2024.003. PMID: 38925868.