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AN INNOVATIVE QUALITY BY DESIGN APPROACH FOR ANALYTICAL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF HYDROCHLOROTHIAZIDE AND OLMESARTAN MEDOXOMIL IN IT'S BULK AND TABLET DOSAGE

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ABSTRACT

The pharmaceutical dosage forms of Hydrochlorothiazideand Olmesartan Medoxomil, a risk-assessed RP-HPLC technique was developed and validated in the current investigation using Quality by Design (QbD). The mobile phase, flow rate, and column temperature are crucial factors in the development of RP-HPLC procedures. Based on these variables, an effective experimental design was made with the help of Central Composite Design (CCD).It was discovered that Hydrochlorothiazide and Olmesartan Medoxomil had retention times of 2.47 and 3.66 minutes, respectively. The Design Expert software 13.0 version, Chemsil ODS column (C18 250 4.6 mm ID and 5µm particle size), Acetonitrile: Phosphate buffer (pH 3.2) in proportion of 70:30 v/v, and flow rate of 1.0 mL/min were used to optimise the chromatographic conditions. With a correlation coefficient (r^2) of 0.9970 and 0.9987 for Hydrochlorothiazide and Olmesartan Medoxomil, respectively, at detection wavelength 261 nm, the linearity of the devised technique was validated over the concentration range of 10–50 µg/mL and 5–25µg/mL. The created method is approved in accordance with ICH rules. Finally, using Design Expert 13.0, the CCD defines the interactions between mobile phase, flow rate, and column temperature at two different levels. Retention time, theoretical plates, and peak

asymmetry were the responses to be seen. In order to better comprehend how the well-known RP-HPLC technology may be applied to meet its intended goals, the factors that affect chromatographic separation are studied in this article. In order to comprehend method variables at different levels, the QbD analytical method development technique was applied.

Keywords: Quality by Design, Hydrochlorothiazide, Olmesartan Medoxomil, RP-HPLC, Design Approach

INTRODUCTION

In order to increase product quality assurance, regulatory flexibility, and continuous improvement, the QbD strategy integrates product and process awareness with quality risk management and their controls **[1-3]**. The ICH Q8 Pharmaceutical Development, ICH Q9 Quality Risk Management, and ICH Q10 Pharmaceutical Quality System standards were followed when developing the QbD technique **[4]**.

Hydrochlorothiazide (HCTZ) chemical name is 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide (Figure1). More sodium ions and the fluid they are in are expelled into the kidney. In order to improve urine production, hydrochlorothiazide acts by preventing the reabsorption of salt and fluid from the urine in the distal convoluted tubule (diuresis). As opposed to this, Olmesartan Medoxomil (OM) is5-methyl-2-oxo-2*H*-1,3-dioxol-4-yl(2-hydroxypropan-2-yl)1*H*-imidazole-5-carboxylate of -2-propyl-1-(4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl]phenyl **(Figure 2)**. OMprevents the angiotensin II's vasoconstrictor effects by specifically preventing angiotensin II from binding to the AT1 receptor in vascular smooth muscles. The way it worked revealed several routes for angiotensin II production. Additionally, it is applied to the management of hypertension **[5-8]**.

According to reported literature survey there are several publications present on HPLC method development. But it has not been generally explored how to use RP-HPLC technique especially for pharmaceutical development by implementation of QbD approach particularly for combination of HCTZ and OM pharmaceutical dosage form. Therefore, the aim of the present research work is to develop and validate RP-HPLC method by using QbD approach for simultaneous determination of HCTZ and OMin pharmaceutical dosage form **[9-12]**. To ensure the efficacy of the developed analytical method during the course of the process, robustness and ruggedness of the QbD based RP-HPLC method **[13]**. However, if a non-rugged or non-robust system is adopted, it may require a lot of time and effort to redesign, revalidate and

retransfer analytical procedures. But, this optimized, developed and validated RP-HPLC method overcomes all these circumstances.

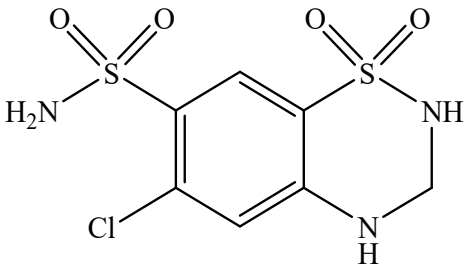

Figure 1: Structure of HCTZ

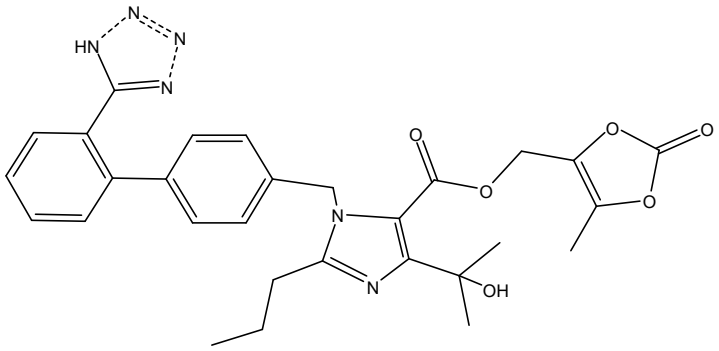

Figure 2: Structure of OM

MATERIALS AND METHODS

Chemicals

OM and HCTZ was procured from Glenmark Pharmaceutical Pvt. Ltd., Sinnar, Nashik. The HPLC grade solvents were used. The other required reagents were also used analytical grade. The marketed formulation of Olmighty-H made by Merck Company was used for assay purpose.

Equipment

UV-VIS spectrophotometer (Shimadzu - 2450) was used for the authentication of the drug sample. WATERS Alliance HPLC-2695 model with UV-VIS Dual Absorbance Detector WATERS-2489 is used for HPLC method development. C_{18} Column (250mm × 4.6mm x 5µm particle size) is used.

Reagents and Solutions preparation

Preparation of Reference Standard Solutions

25mg of each OM and HCTZ were weighed accurately and transferred into separate 25ml capacity of volumetric flask. Then, both drugs were dissolved in ACN solvent and make the volume up to 25ml with the same solvent to obtain 1000µg/ml primary stock solutions A of OM and B of HCTZ, respectively. Further, 100µg/ml sub-stocks

solutions of both drugs were prepared. Finally, 10µg/ml of both drugs were also prepared.

Preparation of Mobile Phase

Mobile phase was prepared by using HPLC grade ACN and Phosphate buffer of pH 3.2 whose pH is adjusted with 1.0% OPA in the ratio 70:30.

Preparation of Diluted OPA

Pipetteout5mlofOPAandtransferreditintoa50mlvolumetricflaskandmadevolumeuptothemark with water.Mixed well andsonicated for5 Minutes.

Preparation of 1.0% OPA in water

Pipette out 1ml of OPA and transferred it into a 100ml volumetric flask and made volumeupto themark with water. Mixed well andsonicated for5 Minutes.

Preparation of 25.00mM phosphate buffer in water

Weighedabout0.34gmofpotassiumdihydrogenorthophosphateanddissolvedin100mlof water pHadjusted to3.2 bydiluted OPA solution.

Method Development

Selection of Detection Wavelength

100µg/ml HCTZ and OM were scanned in the range of 200-400nm and maximum wavelength 261nm was selected for the detection of both drugs **(Figure 3)**.

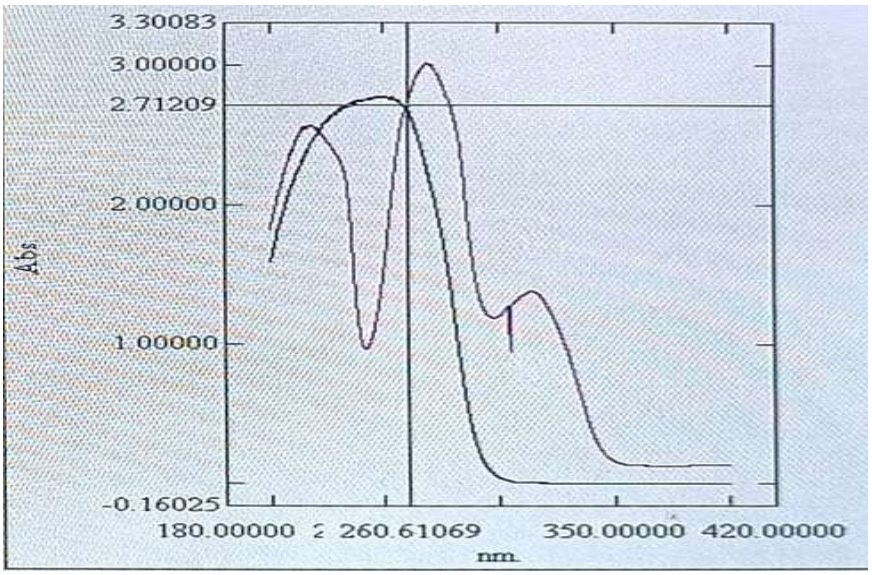

Figure 3: UV spectrum of HCTZ and OM in methanol

Selection of Quality Target Method Profile (QTMP)

QTMP identifies the Critical Quality Attributes (CQAs). QTMP is the target profile of CQAs, which is decided based on the intended use of the method and regulatory requirements. Pharmaceutical products are analysed to ensure that the product meets its intended performance. Product performance comprises of drug safety and efficacy **[14-16]**.

Determination of Critical Quality Attributes (CQAs)

CQAs are the parameters which influence the method performance and it can impact the results of developed RP-HPLC method. The selection of CQAs is based on the techniques used (e.g. HPLC and Gas chromatography) and the method intent (e.g. assay, impurity determination, drug release determination). Theoretical plate, Retention time and peak asymmetry are the selected CQAs for the assay determination method **[17-18]**.

Determination of Critical Method Parameters (CMPs)

CMPs are the methods parameters that have a direct impact on the CQAs. The CMPs needed to be managed for the acceptable response within the range of CQAs. In the present work we have selected mobile phase composition, flow rate and column temperature as CMPs **[15]**.

Factorial design

Three crucial factors composition of the mobile phase, flow rate and column temperature were optimised and chosen using the CCD experimental design. Using a central composite statistical screening design, it was possible to examine the numerous interaction effects and quadratic impacts of the mobile phase composition, flow rate and column temperature on the retention time, theoretical plates and peak asymmetries. The study of multivariable interactions between three variables and process parameters involved in the selection of the factors based on preliminary investigation of column temperature, flow rate and mobile phase composition is shown in **Table 1**. The retention time, theoretical plates and peak asymmetry served as the dependent variables for independent factors that were selected for the trials. With the aid of the Design Expert software (version 13.0), a fractional factorial design was employed with three variables mobile phase composition, flow rate and column temperature at two distinct levels **[15, 19-20]**.

Table 1: Translation of coded levels in actual values

Levelofvariable	Concentrationoffactors		
	Flowrate (mL/min)	Column temperature (°C)	Mobilephasecomposition (v/v)
LowLevel(-1)	0.8	20	65:35
HighLevel(1)	1.2	30	75:25

Evaluation of experimental results and selection of final method conditions

In the first phase, the CCD approach was used to examine the CQAs and assess them. The demonstrated parameters are within the acceptable range. If purposeful changes to the procedure parameters take place it doesn't affect the quality. This assures indicate that the method passes all validation tests. The variables must be adjusted at different levels until the responses are within acceptable limits if the desired response is not obtained from the modelling experiments. The most suitable chromatographic conditions must be optimised with Design Expert tools **[19-20]**.

Risk assessment

A risk-assessed strategy is based on QbD principles and is described in the ICH Q8 and Q9 guidelines. It was used to evaluate the method to examine robustness and ruggedness. The developed features of method and its efficiency and ability to function throughout the duration of the process life are taken into consideration while choosing the optimum final method. For robustness and ruggedness investigations, the parameters of this method or its performance under various conditions were assessed **[18-20]**.

Method Validation

Linearity

The linearity of HCTZ and OM was assessed by analysing five distinct levels of concentration in the range of 10-50µg/ml and 5-25µg/ml, respectively. The calibration curve was created by graphing peak area against concentration on y-axis and x-axis. Resulted values for the correlation coefficient and regression line equation were calculated.

Precision

By measuring six samples of 10µg/ml of HCTZ and 25µg/ml of OM, repeatability was estimated. On the same day, the intraday precision was determined and the interday precision was determined on the following day. The % RSD was observed less than 2.

Accuracy

Recovery studies from commercial formulations at three levels 80%, 100% and 120% in presence of standard and evaluate the accuracy of method. Both HCTZ and OM percentage recoveries were computed. According to ICH recommendations, 98-102% of standard addition was acceptable for percent recovery.

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

The lowest drug concentration that can be precisely detected, distinguished from the background is the LOD and quantified at that concentration is known as the LOQ. The following equations were used to calculate LOD and LOQ in accordance with ICH recommendations.

$LOD = 3.3 \times \sigma/S$

LOQ = 10 × σ/S

Where σ is standard deviation of the y-intercept of the regression line and S is slope of the calibration curve.

Robustness studies

The robustness of the method was calculated by taking different trials through a small change in the flow rate and wavelength. The computed % RSD of peak area acceptability limit was less than 2.

System suitability studies

Six repeat studies of HCTZ and OM were used to determine the system suitability. For standard solutions, the retention time, peak asymmetries and theoretical plates were measured.

RESULTS AND DISCUSSION

The ideal chromatographic conditions were satisfied the system suitability test requirements. The ideal mobile phase contains 70:30 v/v ACN to Phosphate buffer (pH 3.2), 1.0 ml/min flow rate and 25±2℃ column temperature. Additional parameters optimization inside the design space were done using CCD.

Screening Design for Selecting the Critical Method Parameters

Retention time, theoretical plates and peak asymmetry variables were used as CQA for the optimization of RP-HPLC chromatographic conditions. The CMP included the mobile phase ratio 70:30 v/v of ACN to Phosphate buffer (pH 3.2), the flow rate 1.0ml/min and column temperature of 25±2℃. The development of the proposed RP-HPLC method was chosen to use CCD. The optimization of several parameters was mentioned in **Table 2**. We have implemented the quadric design model with 20runs by using response surface methodology and CCD. Applying the suggested CCD experimental design, the three responses retention time, theoretical plates and peak asymmetry were assessed along with the mobile phase composition, flow rate and column temperature. The results of 20 runs were shown in **Table 2**.

Optimization by Response Surface Methodology - CCD

Optimized HPLC parameters and estimated responses are displayed in **Table 3 and Figure 4** as a result of analysis of all responses under various experimental conditions using the Design expert software. By executing the HPLC chromatogram at a specific mobile phase composition, flow rate and column temperature. The observed values for all responses were calculated and compared with standard values **(Figure 4 to Figure 9)**.

Optimization of the Method by Desirability Functions Approach

The optimized chromatographic conditions selected based on the desirability functions approach were mobile phase consisting of ACN: Phosphate buffer pH 4.18 (70:30% v/v) pumped at a flow rate of 0.967 ml/min gave the highest desirability of 1. In the

overlay contour plot shown in **Figure 10**, the flag represents the optimized combination of the three selected independent factors, which gave the maximum desirability.

Table 2: Optimization of parameters for analysis of HCTZ and OM using CCD

Runs	Factor1	Factor2	Factor3	Response1		Response2		Response3	
	ACN	Flow rate (ml/min)	Temperature (°C)	Retention time (Min)		Asymmetry		Theoretical plate	
				HCTZ	OM	HCTZ	OM	HCTZ	OM
1	70	1	25	2.461	3.6	1.1	1.1	3920	5031
2	70	1	33.409	2.2	2.9	1.3	1.3	1583	4962
3	75	0.8	30	2.3	4.3	1.2	1.2	1932	3526
4	65	0.8	30	3.3	4.5	1.4	1.2	2754	4257
5	61.591	1	25	3.1	4.1	1.3	1.4	1380	5234
6	78.409	1	25	2	2.9	1.3	1.2	3291	2673
7	65	0.8	20	4.1	4.4	1.4	1.3	3257	3673
8	75	0.8	20	2.3	4.5	1.2	1.4	3840	4527
9	70	1	25	2.461	3.6	1.1	1.1	3920	5031
10	75	1.2	20	1.9	3.2	1.4	1.2	1745	4527
11	70	1	25	2.461	3.6	1.1	1.1	3920	5031
12	70	1	25	2.461	3.6	1.1	1.2	3920	1256
13	70	1	16.591	3.4	4.6	1.3	1.3	3211	2314
14	70	1	25	2.461	3.6	1.1	1.1	3920	5031
15	70	1	25	4.1	3.6	1.4	1.1	3257	5031
16	70	1.33636	25	2.2	2.8	1.4	1.2	1865	3452
17	70	0.663641	25	3.1	4.8	1.4	1.5	4389	5263
18	75	1.2	30	2.12	3.4	1.2	1.3	2250	4253
19	65	1.2	20	2.5	3.8	1.2	1.4	1649	4352
20	65	1.2	30	2.3	3.2	1.2	1.3	2537	4231

Table 3: Obtained solution for optimized formulation

ACN: Phosphate buffer (v/v)	Flow rate (ml/min)	Temp. (°C)	Retention time		Asymmetry		Theoretical plate	
			HCTZ	OM	HCTZ	OM	HCTZ	OM
70:30	1	25	2.461	3.6	1.1	1.1	3920	5031

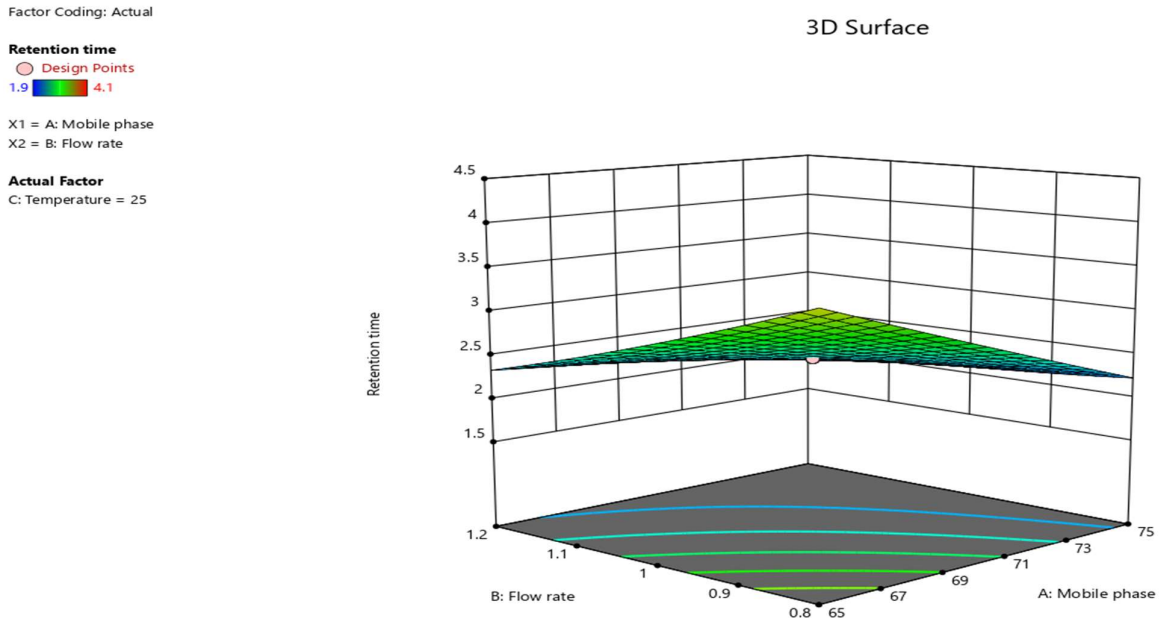

Figure 4: 3D Response plot of retention time against flow rate and mobile phase composition for HCTZ

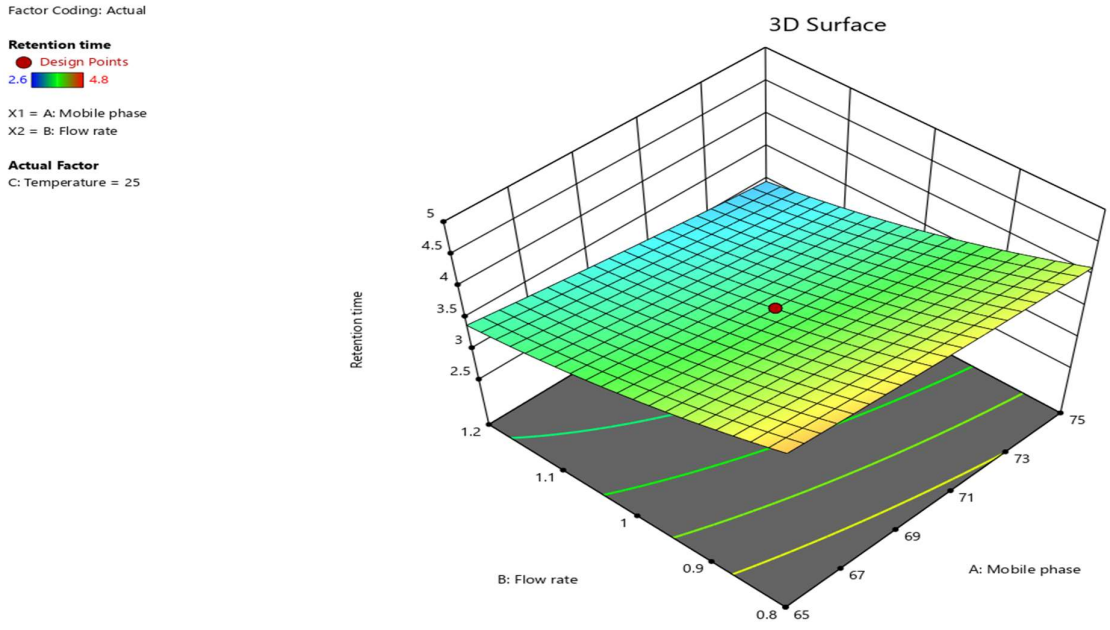

Figure 5: 3D Response plot of Retention time against flow rate and mobile phase composition for OM

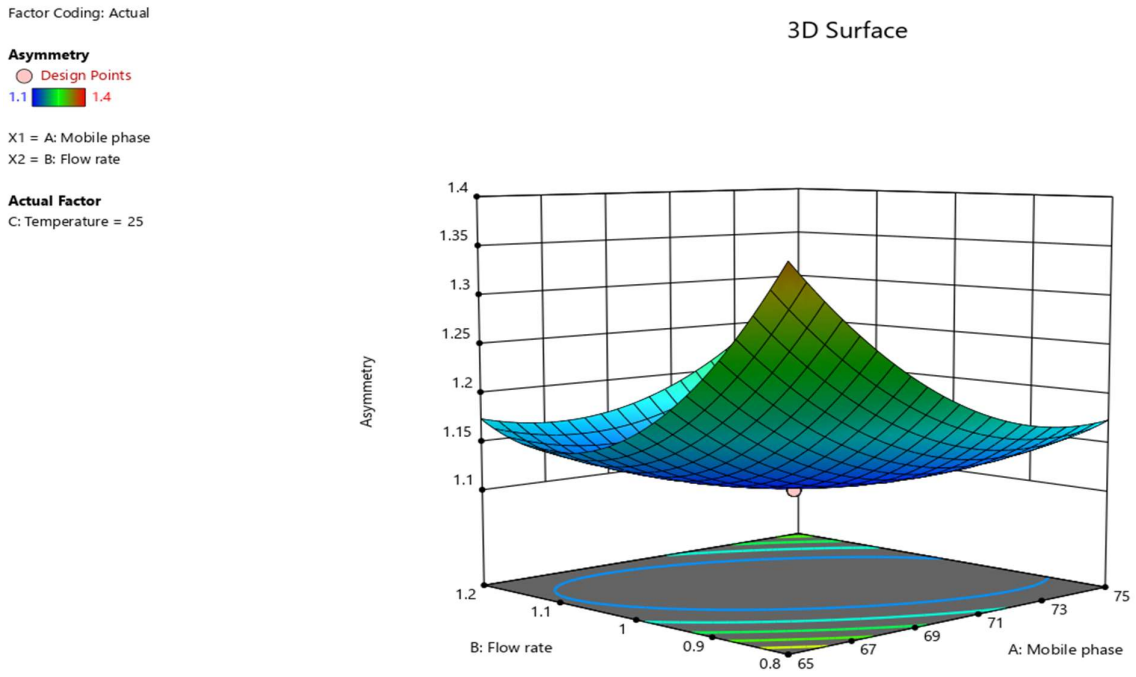

Figure 6: 3D Response plot of asymmetry against flow rate and mobile phase composition for HCTZ

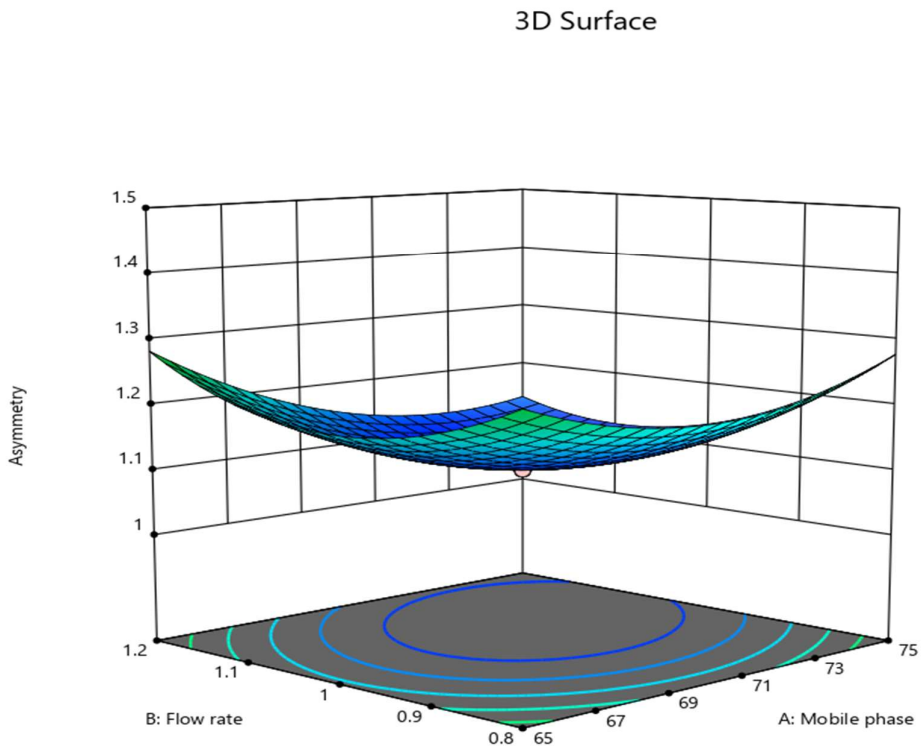

Figure 7: 3D Response plot of asymmetry against flow rate and mobile phase composition for OM

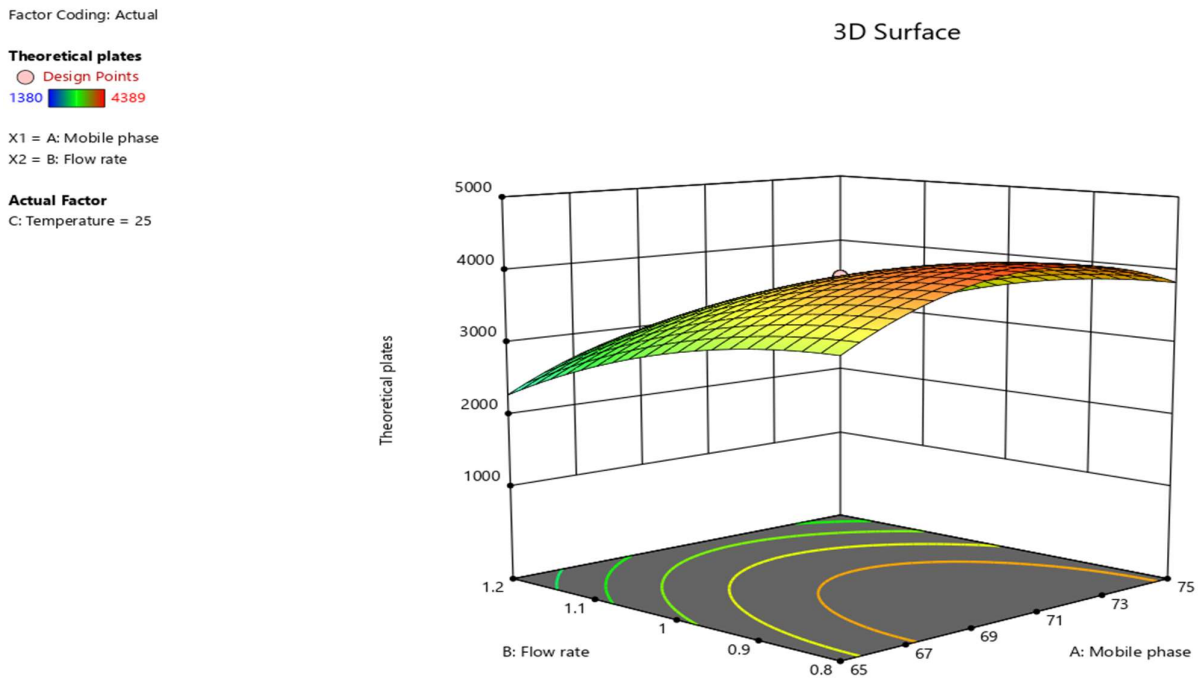

Figure 8: 3D Response plot of Theoretical plates against flow rate and mobile phase composition for HCTZ

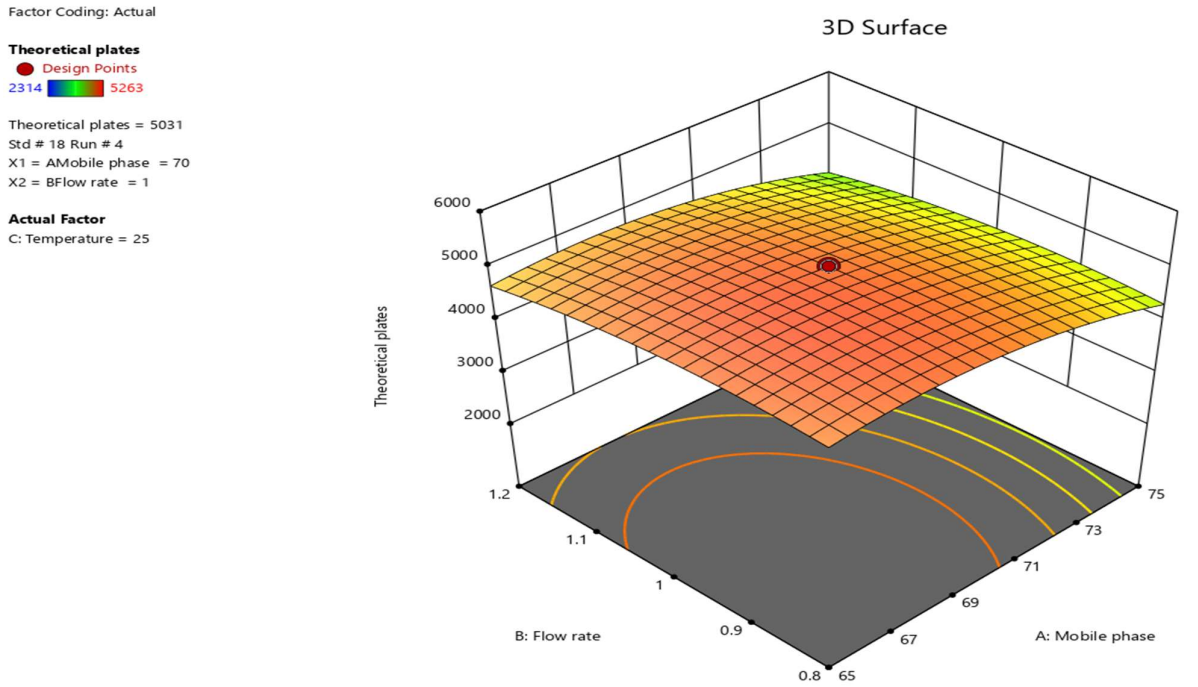

Figure 9: 3D Response plot of theoretical plates against flow rate and mobile phase composition for OM

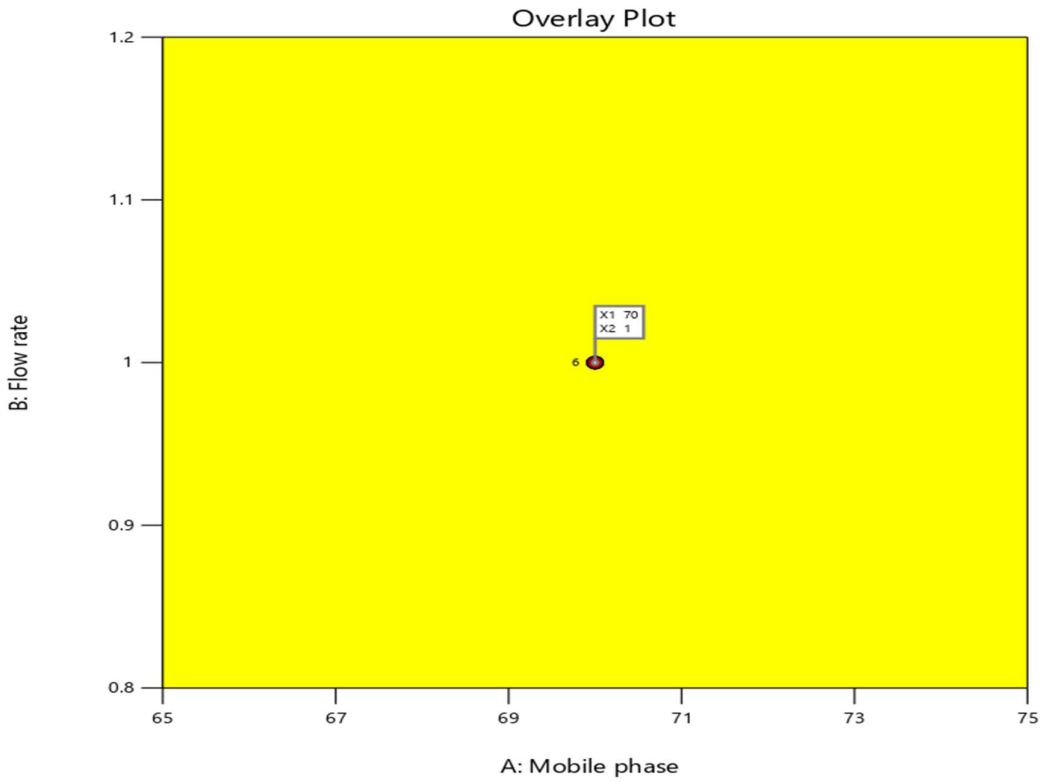

Figure 10: Design space for DOE

Optimized Chromatographic Conditions

Column: Chemsil ODS column (C_{18} 250 × 4.6mm, 5µ)

Mobile phase: ACN: Phosphate buffer pH 3.2 (70:30%v/v)

Buffer pH: 3.2

Flow rate: 1.0ml/min

Wavelength: UV detection at 261nm

Column temperature: 25°C

Injection volume: 20µl

Run time: 10min

Method Validation

A representative chromatogram was subjected to the system suitability test for examination of various parameters such as retention time, theoretical plates and peak asymmetry. The retention time was found to be 2.47min for HCTZ and 3.66min for OM **(Figure 11)**. The theoretical plates were found to be 4386 for HCTZ and 5581 for OM. The peak asymmetry was found to be same 1.1 for both drugs. The % RSD of six replicate injections of HCTZ and OM was found to be 0.53 and 0.68, respectively.

Linearity

The constructed calibration curve of HCTZ and OM was linear in the concentration range of 10-50 and 5-25µg/ml, respectively shown in **Table 4**. The regression equations of the calibration curve for HCTZ and OM were found to be y = 47669 x + 101766 and y = 49479 x + 257199 with its 0.9970 and

0.9987 correlation coefficients, respectively when graph was plotted with peak area verses concentration **(Figure 12 and Figure 13)**.

Precision

The % RSD for six times repeatability of HCTZ and OM measured at concentration 10µg/ml and 25µg/ml and it was found to be 0.967 and 1.33, respectively. The results of interday and intraday precisions were shown in **Table 5** and **Table 6**. The developed method was found to be precise as % RSD was found to be less than 2.

Accuracy

Recovery study was performed to determine the accuracy. Sample solutions were made by spiking at three different levels viz 80, 100 and 120%. The percent recovery results were obtained using HPLC technique and reported in **Table 7**. According to ICH Q2 (R1) guidelines, the developed method was accurate and the % recovery was found to be in between 98 and 102%.

Robustness studies

A 100 µg/ml solution of HCTZ and OM was utilised for robustness studies. The robustness was investigated by making a small change to the intrinsic methods of flow rate and wavelength parameters. By changing the flow rate and wavelength, the resultant % RSD was less than 2.

LOD and LOQ

The LOD and LOQ for HCTZ based on standard deviation of slope and intercept were found to be 3.47µg/ml and 10.51µg/ml respectively. Meanwhile, the LOD and LOQ of OM were found to be 1.14µg/ml and 3.46µg/ml, respectively.

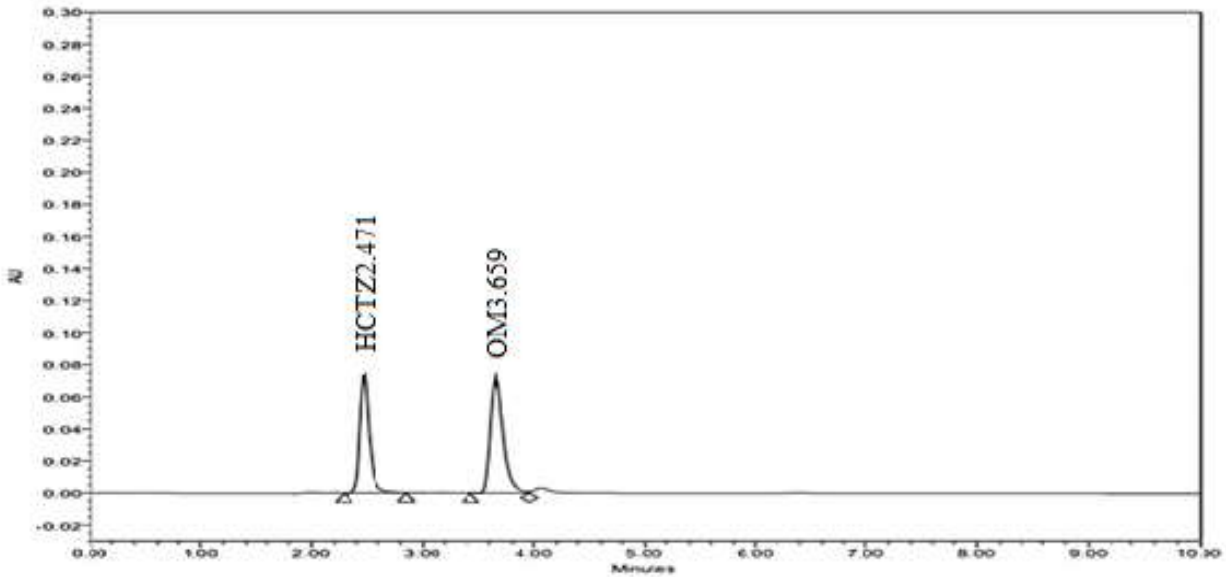

Figure 11: Typical chromatogram of standard HCTZ and OM

Table 4: Linearity of HCTZ and OM

Sr. No.	Concentration (µg/mL)		Peak area	
	HCTZ	OM	HCTZ	OM
1.	10	5	625244	504024
2.	20	10	989845	767773
3.	30	15	1527008	981442
4.	40	20	2027008	1237616
5.	50	25	2490132	1506084

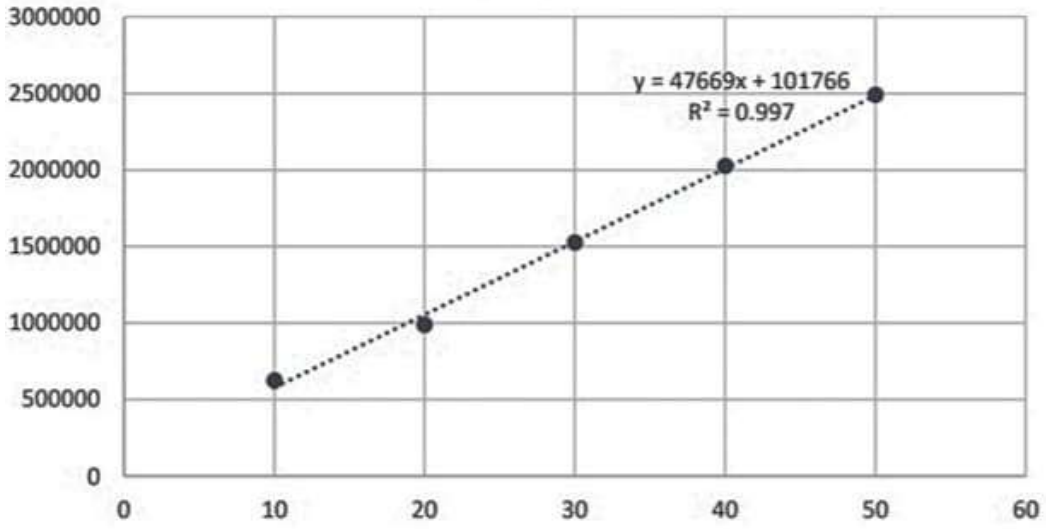

Figure 12: Linearity of HCTZ

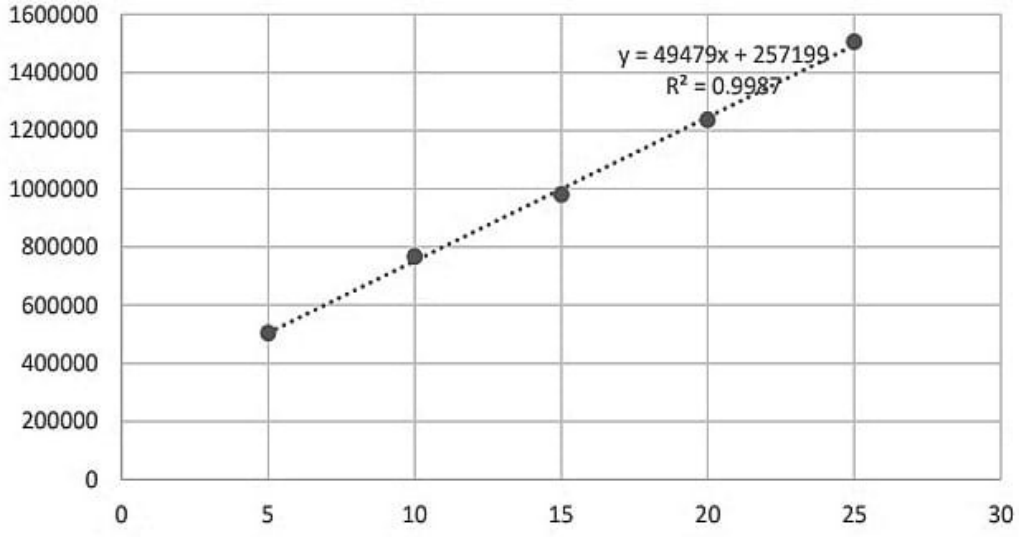

Figure 13: Linearity of OM

Table 5: Data for intraday precision of HCTZ and OM

Sample	Concentration(µg/mL)		Area	
	HCTZ	OM	HCTZ	OM
Sample 1	10	25	625244	1556284
Sample2	10	25	622548	1538020
Sample3	10	25	611965	1504123
Sample4	10	25	625847	1506000
Sample5	10	25	621245	1515055
Sample6	10	25	613546	1522112
	Mean		620066	1523599
	SD		5931.6	20201.6
	% RSD		0.967	1.33

Table 6: Data for interday precision of HCTZ and OM

Sample	Concentration (µg/mL)		Area	
	HCTZ	OM	HCTZ	OM
Sample 1	10	25	622145	1625142
Sample2	10	25	622145	1612445
Sample3	10	25	612546	1592546
Sample4	10	25	625486	1589642
Sample5	10	25	625879	1645698
Sample6	10	25	614536	1632544
Mean			620456	1616336
SD			5622	22343
% RSD			0.97	1.38

Table 7: Recovery of HCTZ and OM

Sr. No.	Concentration levels (%)	Average recovery (%)		SD		RSD (%)	
		HCTZ	OM	HCTZ	OM	HCTZ	OM
1	80	100.60	100.28	0.951438	1.060864	0.949	1.058
2	100	100.49	99.79	0.82203	1.292788	0.824	1.296
3	120	99.49	99.82	0.835005	0.743236	0.837	0.745

Table 8: Results of the validation parameters

Sr.No.	Parameters	Sub parameters	HCTZ	OM
1	Linearity	Linearity range (µg/ml) Correlation coefficient Regression equation	10-50 0.9970 y=47669x+ 101766	5-25 0.9987 y=49479x+257199
2	Precision (% RSD of peak area	Interday precision Intraday precision	0.970 0.967	1.38 1.33
3	Accuracy(% recovery)	80,100,120 levels	99.49-100.60	99.79-100.28
4	Sensitivity	LOD (µg/ml) LOQ (µg/ml)	3.47 10.51	1.14 3.46
5	Robustness(% RSD of peak area)	Flow rate (0.2ml/min) Wavelength (3nm)	0.8 0.9	0.6 0.8
6	System suitability	Retention time (min) Assymetry Plate count	2.47 1.26 4378	3.66 1.40 5534

CONCLUSION

In the present research study, a QbD strategy for RP-HPLC method development has been described. The analytical QbD concept was applied for the development of RP-HPLC methods of HCTZ and OM. In order to identify the optimal system and the final design space, a multivariant analysis of a number of critical process parameters such as three variables flow rate, mobile phase composition and column temperature at two different levels was carried out. In addition, use of CCD, the interrelationships were investigated and optimised at two levels. This strategy provides proper information and understanding for the development and optimization of chromatographic conditions. The acceptance criteria of every parameters were determined and verified it occurs within the limit of ICH guidelines. Finally, the verified results of simultaneously determining HCTZ and OM was linear, precise, accurate and robust. With a greater understanding of the method variables, there is a lower risk of failure during method validation. The automated QbD method development approach using the Design Expert software was provided a better performance. The statistical analysis of data indicates that, the method is reproducible, selective, accurate and robust. This method will be used further for routine analysis for quality control in pharmaceutical industry.

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