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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TELMISARTAN AND HYDROCHLOROTHIAZIDE IN IT'S BULK AND TABLET DOSAGE FORM USING ANALYTICAL QUALITY BY DESIGN APPROACH

JAGDALE AS*, KAPSE OK, SONAWANE MS, THAKUR PV AND BAWISKAR PR

Department of Pharmaceutical Chemistry, MVP Samaj's College of Pharmacy, Nashik-422004, (M.S.), India

***Corresponding Author: Dr. Amol S. Jagdale: E Mail: asjagdale@mvpcpn.edu.in**

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ABSTRACT

This research describes the development and validation of an RP-HPLC method for assaying Hydrochlorothiazide (HZ) and Telmisartan (TEL). Optimization was done by response surface methodology, applying a three-level Box Behnken design. Three factors selected were mobile phase, flow rate, and resolution. Chemsil C18 (4.6 x 250mm, 5µm) was used for chromatography. The mobile phase was 55:45% v/v OPA:ACN (pH 2.5 adjusted with 0.1% TEA). All events were detected at 282 nm using isocratic elution at 1.2 mL/min. The retention time of HZ and TEL were found to be 2.6 min and 5.5 min, respectively. The specificity, linearity, accuracy, precision, limit of detection, the limit of quantitation, and system suitability were examined. HZ and TEL had linear calibration curves between 20-60 µg/mL, and their correlation coefficients (r2) were 0.999. The relative standard deviation (RSD) was less than 2%, while HZ and TEL recoveries were 98-100%. HZ and TEL have detection and quantitation limits of 0.79597 and 2.41203 µg/mL, respectively. The RP-HPLC method that was produced and assessed takes less time and can be used regularly in the industry for quality control and analysis of bulk drugs and products.

Keywords: RP-HPLC, Hydrochlorothiazide, Telmisartan, Development, QbD, Validation

INTRODUCTION:

Hydrochlorothiazide is 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide and Telmisartan is 4-[(1, 40-dimethyl-20-propyl [2, 60-bi-1Hbenzimidazol]-10-yl) methyl]- [1, 10-biphenyl]-2-carboxylic acid. The multi-component dosage forms have a lot of value since more patients are willing to take them, they have numerous actions, and they provide faster relief from more than one ailment. Telmisartan (TMS) is an angiotension receptor blocker that has a lengthy duration of action and the longest half-life of any ARB. It has high affinity from the angiotensin II type 1-receptors and has a high angiotensin II type 1-receptor binding affinity. In addition to inhibiting the Renin-Angiotensin System (RAS), TMS also functions as a selective modulator of the peroxisome proliferator-activated receptor gamma (PPAR-), which is an essential regulator of insulin and glucose metabolism. Hydrochlorothiazide, often known as HCZ, is a diuretic (sometimes known as a water pill), which means that it helps manage blood pressure by removing excess salt and water from the body. According to a literature review, there are few publications on UV-visible spectroscopy and HPLC, but no one has used Quality by Design. To ensure process consistency throughout the product lifecycle, simple validated RP-HPLC methods for the determination of telmisartan and hydrochlorothiazide in pharmaceutical dosage forms must be established using the Quality by Design (QbD) approach as per ICH Q8 (R2) guidelines.

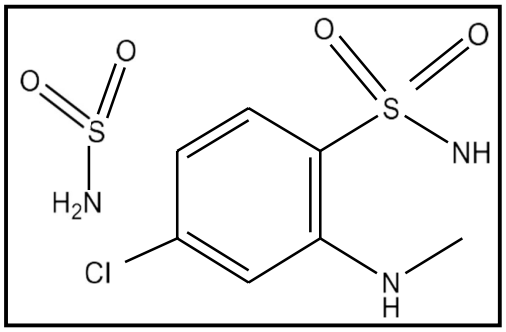


Figure 1: Chemical Structure of Hydrochlorothiazide

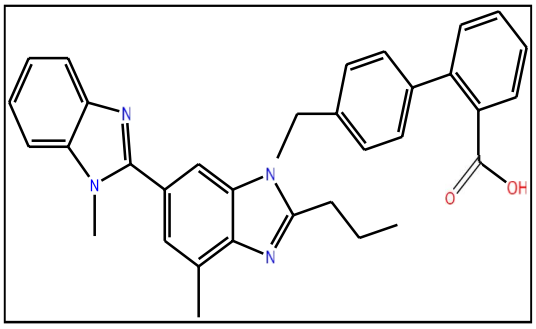


Figure 2: Chemical Structure of Telmisartan

METHODS:

Materials and Reagents:

Shreya life science Pvt. Ltd. Aurangabad donated Hydrochlorothiazide **(Figure 1)** and Virchow pvt. Ltd. Donated Telmisartan **(Figure 2)**. Alpha chemicals provided HPLC grade methanol, acetonitrile, orthophosphoric acid (OPA), HPLC grade water.

Instrumentation and Software:

An Agilent HPLC system contains chromatographic system Waters 1525 Binary

Pump and Waters 2489 UVvisible detector. For data collection and processing, the chromatograms were registered using empower software on a Windows-based computer system. The Column: Chemsil ODS-C18 column Column Dimension: 250mm × 4.6mm, 5µm were used.

Qbd Software:

Design Expert® software (Design Expert trial version 13.0.5.0; 64-bit, State-Ease Inc., Minneapolis, MN, USA).

PREPARATIONS OF SOLUTIONS

Preparation of standard stock solution:

The standard solution was made by dissolving 10 mg of hydrochlorothiazide and telmisartan in a 10mL clean and dry volumetric flask, then adding approximately 7mL of methanol to fully dissolve it and fill the flask to the mark with methanol (1000µg/mL) and allow to sonicate it.

Sample preparation:

10mg (equivalent weight) of hydrochlorothiazide and telmisartan was correctly weighed and transferred to a 10mL volumetric flask. 7 mL diluent was added and sonicated to fully remove it. Using diluent, dilute the mixture by another 10 to 20 mL.

Preparation of 0.1% OPA in water:

0.1mL of OPA was transferred into a 100 mL volumetric flask and diluted up to the mark with water. Mixed well and sonicated for 15 Minutes.

Preparation of diluted TEA:

Pipette 1 mL of OPA into a 10-mL volumetric flask and top up with water to reach the desired amount. Sonicate for 5 min after thoroughly mixing.

Determination of detection wavelength:

Between 200 and 400 nm, the standard solution was scanned. As shown in **Figure 3**, the wavelength of maximum absorption for drug was determined to be 282 nm.

Table 1: Experimental results and selected method conditions

Parameter/condition	Description
Injection volume	6µL
Wavelength	282 nm
Mobile phase	OPA:CAN (55:45%v/v)
Program	Isocratic
Flow rate	1.2mL/min
Column oven temp	25 °C
Run time	8 min
Buffer	0.1% OPA in water. Adjusted with TEA.

METHOD DEVELOPMENT BY QBD APPROACH

Application of design of experiments for method optimization:

Thus, 3^3 randomized response surface design with a Box-behnken design was used with 17 trial runs to study the impact of three factors on the two key response variables. In this design 3 factors were evaluated, each at 3 levels, and experimental trials were performed at all 3 possible combinations. The Mobile Phase composition (X1), flow

rate (X2), Buffer pH (X3) was selected as independent variables and retention time (RT) and Resolution were selected as dependent variables. The resulting data were fitted into Design Expert® software (Design Expert trial version 13.0.5.0; 64-bit, State-Ease Inc., Minneapolis, MN, USA) software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to determine the influence of mobile phase composition, flow rate, & buffer pH on dependent variables.

ANALYSIS OF THE SAMPLE:

Hydrochlorothiazide and Telmisartan drug (API): The drug sample solution was prepared by diluting 10 mg of HZ and TEL API into a 10mL volumetric flask, adding 7mL of methanol to fully melt it by sonication, and then adjusting the volume with solvents (1000µg/mL). Filtered through a suitable filter, and a sufficient amount of the sample solution was discarded. Using methanol (100µg/mL), dilute 1 mL of the filtrate solution to 10 mL.

Preparation of Test solution:

10 mg of TEL and 10 mg of HZ was transferred into a 10 mL volumetric flask separately. About 5 mL of diluent was added and was shaken vigorously to disperse the material completely followed by sonication for 10-15 min, cooled to room temperature, make up to mark with diluent and mixed well. 0.4 mL and 0.125 mL of the above filtrate was further diluted up to 10 mL with diluent to obtain final concentrations 40 µg/mL and 12.5 µg/mL respectively.

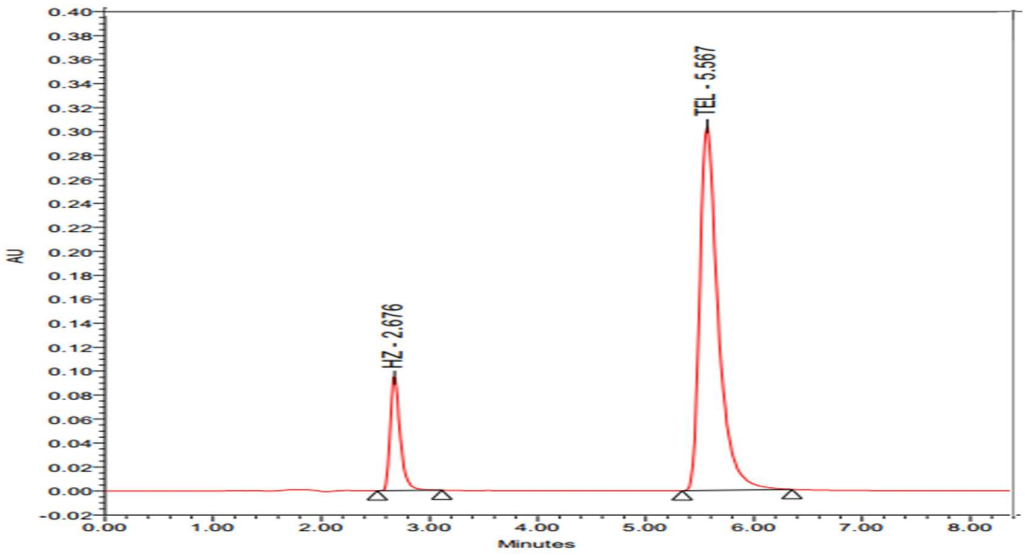


Figure 3: Typical chromatogram obtained from optimized mobile phase

Table 2: Analytical data of optimized run

Drug	R.T. (min)	Area	Theoretical Plate (USP)	USP Tailing	Resolution
HZ	2.671	612100	4160	1.5	
TEL	5.554	1836300	5482	1.4	10.74

METHOD VALIDATION:

The developed method for estimating hydrochlorothiazide and telmisartan was validated for the following parameters using ICH Q2 (R1) guidelines.

Specificity

To demonstrate the method's precision, the following solutions will be prepared and injected (double-checked the peak purity). It includes Blank (methanol as a diluent), HZ and TEL standard solution, HZ and TEL sample solution, Placebo treatments.

Linearity and range

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of an analyte in samples within a given range. The region is graphically plotted. Curve fitting percentages are measured.

Accuracy (%recovery)

The accuracy of the analytical technique expresses the closeness of agreement between the value which is acknowledged either as a conventional true value or an acceptable reference value and the value of the value found. Accuracy will be conducted in the region of 80 percent to 120 percent of the working concentration. The solution for each accuracy level will be prepared in triplicate. percent Recovery computed for each sample.

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. The precision of an analytical method is usually expressed as a standard deviation or relative standard deviation. Precision is of two types, Repeatability and Intermediate precision. It is performed on an API sample. Prepare six different test solutions of the 100% test concentration from the same sample matrix. Inject duplicate injections of each test solution.

SENSITIVITY

Limit of Detection (LOD):

The lowest conc. of the analyte in the sample that the method can detect but not necessarily quantify under the stated experimental conditions simply indicates thatthe sample is below or above a certain level. Limit tests prescribed as a percentage oras parts per million. The limit of detection

will not only depend on the procedure of analysis but also the type of instrument.

LOD= 3.3 (SD)/S

Limit of Quantitation (LOQ):

The limit of quantitation (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. It is expressed as the conc. of analyte (e.g., percentage, parts per billion) in the sample. The S/N ratio should not be less than 10 and RSD ≤ 3%.

LOD= 3.3 (SD)/S

RESULTS

Optimization of mobile phase:

Various trials were performed as methanol : water (70:30% v/v), ACN : water pH 4.5 (60:40% v/v), phosphate buffer (pH 3) : methanol (60:40% v/v), ammonium formate (pH 3.5) : methanol (65:35% v/v), OPA (pH 4.5) : ACN (50:50% v/v), finally got HZ was eluted at 2.6 min and TEL at 5.5 min. Proper resolution was obtained with sharp peaks and no tailing obtained at OPA (pH 2.5): ACN (55:45% v/v). Typical chromatogram of optimized run is shown in **Figure 3**. Analytical data for typical chromatogram of optimized run are shown in **Table 2**.

Table 3: Design summary for optimization

Study type	Design type	Design model	Total runs
Response surface	Central composite design	Central composite design	17

Table 4: Obtained solution for optimized formulation

Runs	Factor 1 A: ACN	Factor 2 B: Flow rate	Factor 3 C: pH	Response 1 RT	Response 2 RT	Response 3 Rs
1	45	1.2	2.5	2.68	5.73	11.49

Optimization of various parameters for analysis of HZ and TEL using HPLC (by using Box-behnken design):

Design summary for optimization is given in **Table 5**. Obtained solution for optimized formulation.

System suitability test (SST):

System suitability is a Pharmacopeial requirement and is used to verify, whether the chromatographic system i.e. The Instrument and Analyst is adequate for analysis to be done. The tests were performed by collecting data from Five replicate injections of standard drug solution and the results are recorded. Typical chromatogram of SST for HZ and TEL is shown in **Figure 19** Analytical data of system suitability test are given in table 6.

Specificity:

Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present. The following solutions were prepared and injected to prove the

specificity nature of the method. Result of specificity shown in **Table 7**.

A. Blank (Methanol as a diluent)
B. Standard solution
C. Sample solution
D. Placebo solutions

Accuracy (%recovery):

%Recovery was found well within acceptance range (98.00 to 102.0%) at all three levels. Result and statistical data of accuracy are given in **Table 8**.

Precision:

%RSD for 12 samples (precision and intermediate precision samples) NMT 2.0%. The %RSD of method precision is (intraday- 0.5 & 1.3) (interday- 1.04&1.3) Therefore, the HPLC method for the determination of HZ and TEL is precise. Analytical data of both precision of HZ and TEL is given in **Table 9**.

Linearity

From the calibration curve, we had to conclude that HZ and HZ shows linear response in the range of 20-60 µg/mL. The regression value was found well within the limit. Result and statistical data of linearity of HZ and TEL are given in table 10. Linearity graph of HZ and TEL is shown in **Figure 20 & 21**.

Sensitivity:

It may be calculated based on the standard deviation (SD) of the response and slope of the curve (S). Result of detection limit is given in **Table 14**. Calibration curve of HZ and TEL for LOD and LOQ is given in **Table 14**.

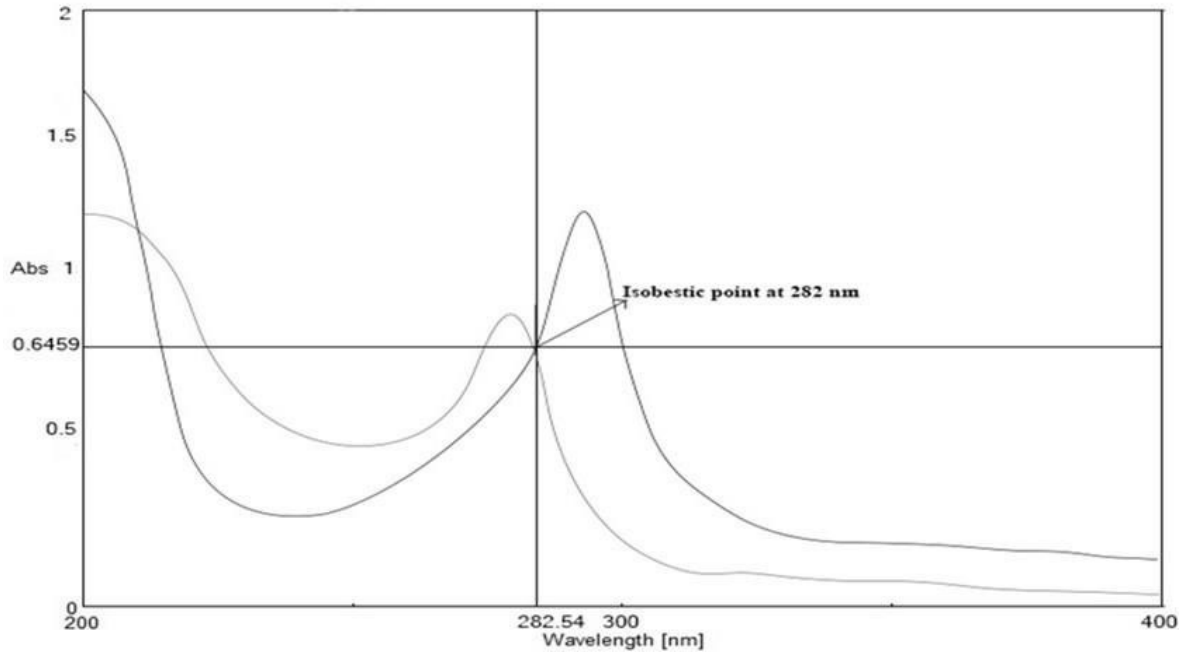


Figure 4: Ultraviolet (UV) spectroscopy-Isobestic point- TEL and HZ

Table 5: Layout of actual Design of DOE

Runs	Factor1	Factor 2	Factor3	Response1	Response2	Response3
1	45	1.25	2.5	2.68	5.73	11.49
2	45	1.5	2.4	2.4	5.11	10.45
3	50	1.25	2.6	2.5	5.61	11.2
4	50	1	2.5	2.8	5.81	12
5	40	1	2.5	3.1	5.85	13.1
6	40	1.25	2.6	2.9	5.92	11.88
7	40	1.25	2.4	2.8	5.72	11.58
8	45	1.25	2.5	2.68	5.73	11.49
9	45	1	2.4	2.7	5.83	11.72
10	45	1.25	2.5	2.68	5.73	11.49
11	40	1.5	2.5	2.9	5.11	11.5
12	45	1.25	2.5	2.68	5.73	11.49
13	45	1.5	2.6	2.71	5.75	11.5
14	45	1	2.6	2.8	5.82	11.52
15	45	1.25	2.5	2.68	5.73	11.49
16	50	1.5	2.5	2.5	5.12	10.34
17	50	1.25	2.4	2.4	5.45	10.21

Graphical Presentation: Retention Time 1

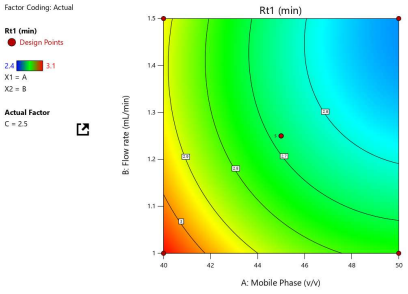


Figure 5

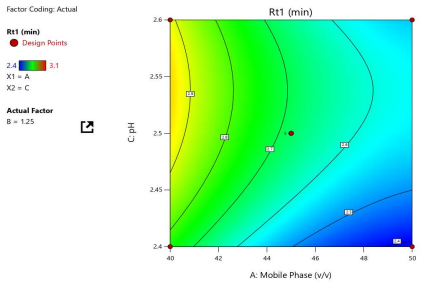


Figure 6

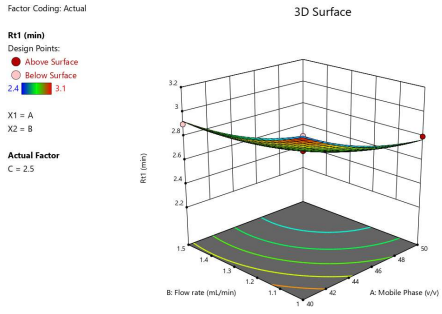


Figure 7

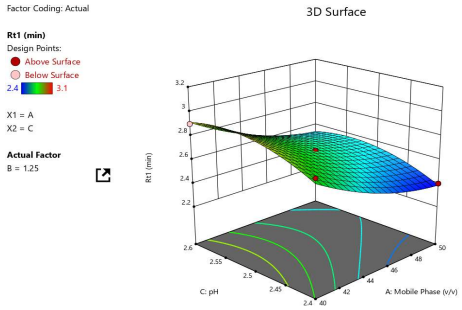


Figure 8

Graphical Presentation: Retention Time 2

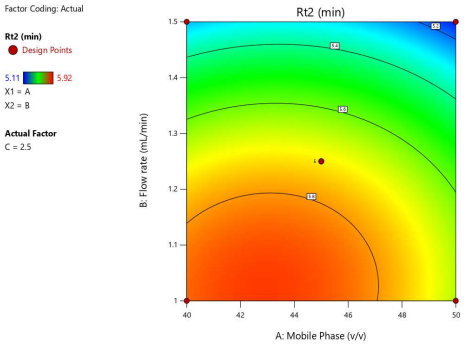


Figure 9

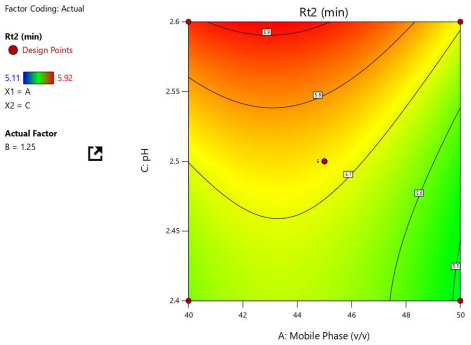
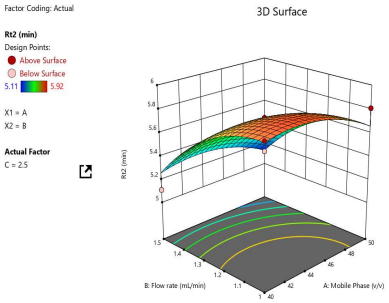


Figure 10



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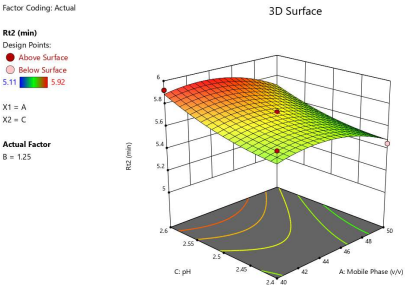


Figure 12

Graphical Presentation: Resolution

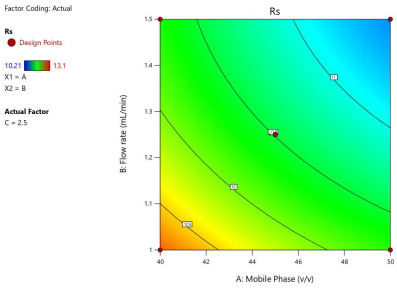


Figure 13

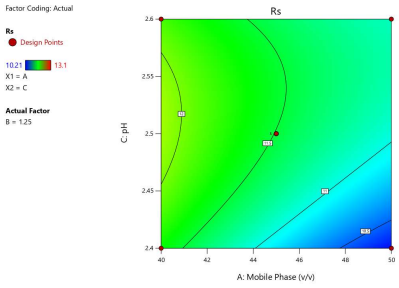
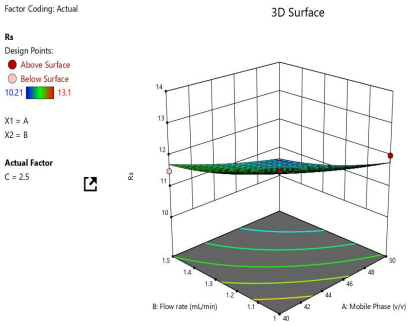


Figure 14



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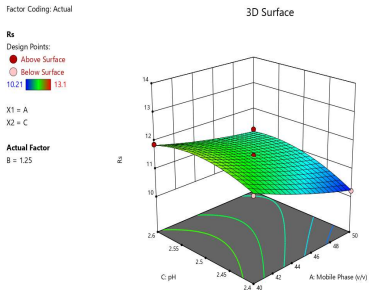


Figure 16

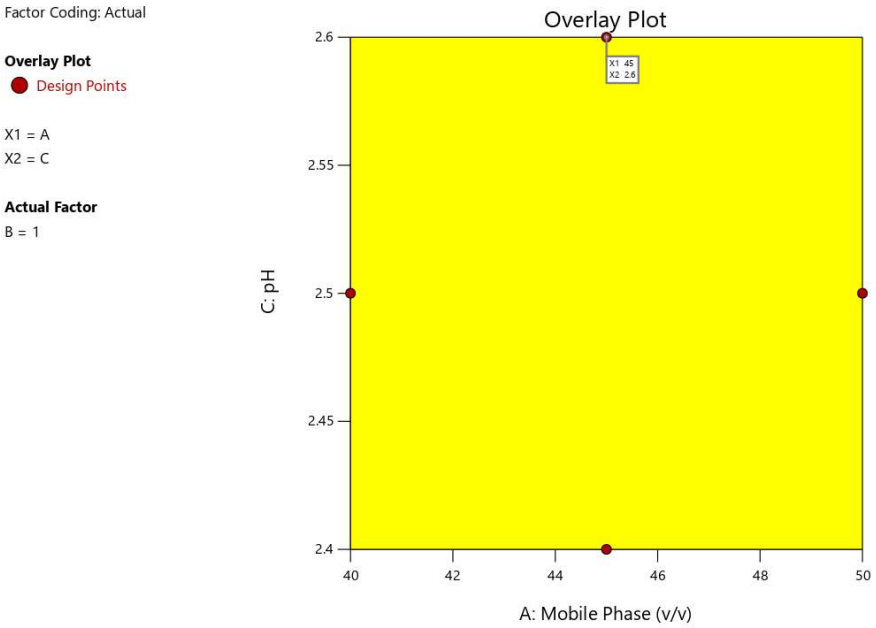


Figure 17: Design Space for DOE

Figure Legends

Fig. 5: Contour plot for retention time (min) against mobile phase and Flow rate (AB)
Fig. 6: Contour plot for retention time (min) against mobile phase and pH (AC)
Fig. 7: Response plot for retention time (min) against mobile phase and Flow rate (AB)
Fig. 8: Response plot for retention time (min) against mobile phase and pH (AC)
Fig. 9: Contour plot for retention time (min) against mobile phase and Flow rate (AB)
Fig. 10: Contour plot for retention time (min) against mobile phase and pH (AC)
Fig. 11: Response plot for retention time (min) against mobile phase and Flow rate (AB)
Fig. 12: Response plot for retention time (min) against mobile phase and pH (AC)
Fig 13: Contour plot for Resolution against mobile phase and Flow rate (AB)
Fig. 14: Contour plot for Resolution against mobile phase and pH (AC)
Fig. 15: Response plot for Resolution against mobile phase and Flow rate (AB)
Fig. 16: Response plot for Resolution against mobile phase and pH (AC)
Fig. 17: Design Space for DOE

Table 6: Analytical data of system suitability test

Parameter	Acceptance criteria	Result
%RSD	NMT 2.0%.	1.14 & 0.55
Theoretical plates	More than 2000	4204 & 7429
Tailing factor	NMT 2.0	1.23 & 1.3

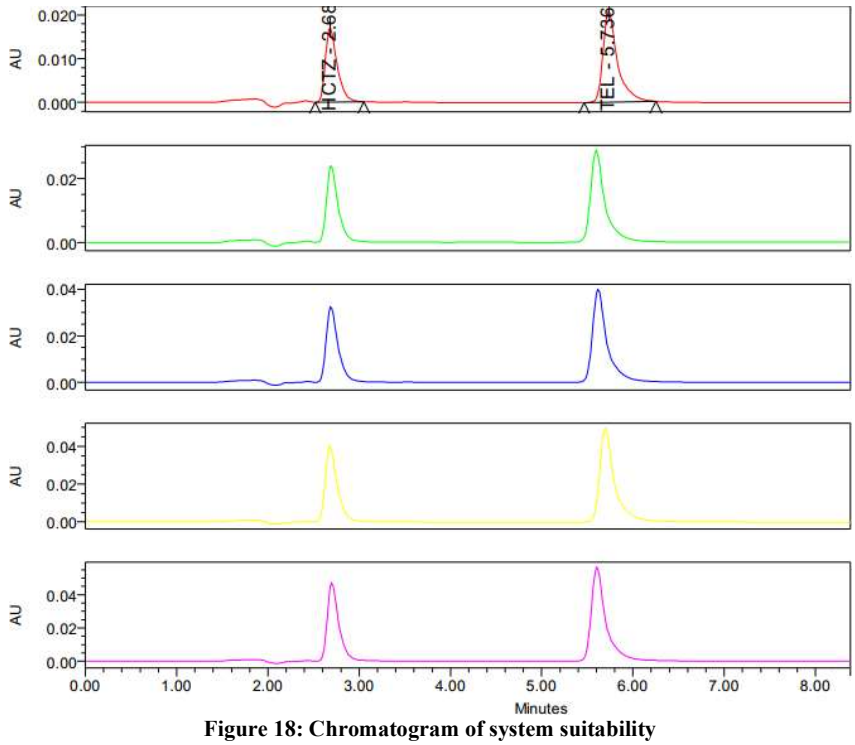


Figure 18: Chromatogram of system suitability

Table 7: Results of specificity

Description	Description	Acceptance criteria	Conclusion
Blank	No interference at R.T. of HZ & TEL in blank	No interference at R.T	Developed chromatographic method passed the criteria for specificity.
Standard solution	Peak purity was 0.998	Peak purity: NLT 0.95	
Sample solution	Peak purity was 0.998	Peak purity: NLT 0.95	
Placebo	No interference at R.T. of HZ & TEL in placebo	No interference at R.T.	

Table 8: Result and statistical data of accuracy of HZ and TEL

Conc. Taken (22.5ppm) 80%		Conc. Taken (25ppm) 100%		Conc. Taken(27.5ppm) 120%	
Area	Conc. Found (ppm)	Area	Conc. Found (ppm)	Area	Conc. Found (ppm)
178804	22.49551387	199917	25.73365439	209917	27.2673732
169989	21.14354074	197863	25.41862855	217863	28.4860662
184093	23.30669775	189953	24.20545697	212953	27.7330102
Mean Conc.	**22.31525079**		**25.11924664**		**27.82881653**
SD of Conc.	**1.09278687**		**0.806888459**		**0.614969325**
%Recovery	**99.17889239**		**100.4769865**		**101.1956965**

Conc. Taken (72ppm) 80%		Conc. Taken (80ppm) 100%		Conc. Taken (88ppm) 120%	
Area	Conc. Found (ppm)	Area	Conc. Found (ppm)	Area	Conc. Found (ppm)
740554	71.81124591	820554	79.90619972	918620	89.82919647
722733	70.00799377	838620	81.73424267	910534	89.01099902
756630	73.43792688	819653	79.81503031	906630	88.61596527
Mean Conc.	**71.75238885**		**80.48515756**		**89.15205359**
SD of Conc.	**1.715723873**		**1.082699478**		**0.618793004**
%Recovery	**99.65609563**		**100.606447**		**101.3091518**

Table 9: Data of precision of HZ & TEL

Parameters	Intraday precision	Interday precision	Acceptance criteria	Conclusion
Mean	**363577.167**	**553031**	**% RSD for the six samples NMT 2.0**	**HPLC method for the Determination of HZ is Precise.**
SD	**2116.23150**	**7583.244345**		
%RSD	**0.58205841**	**1.371215058**		

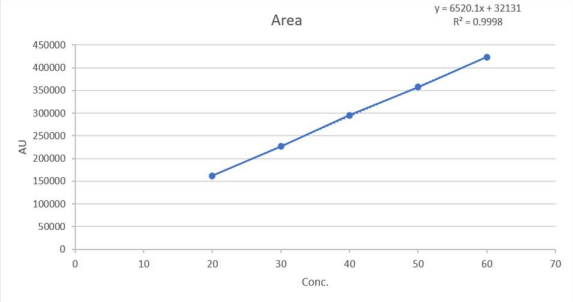
Parameters	Intraday precision	Interday precision	Acceptance criteria	Conclusion
Mean	**384023.167**	**553031**	**% RSD for the six samples NMT 2.0**	**HPLC method for the Determination of TEL is Precise.**
SD	**4000.31596**	**7583.24435**		
%RSD	**1.0416861**	**1.37121506**		

Table 10: Result and statistical data of linearity of HZ & TEL

Level	Sample name	Conc. of Std. Solution(µg/mL)	RT	Area
1	Std 1	20	2.682	162180
2	Std 2	30	2.693	226899
3	Std 3	40	2.69	295331
4	Std 4	50	2.681	357274
5	Std 5	60	2.702	422999
	Avg Area			292936.6

Level	Sample name	Conc. of Std. Solution(µg/mL)	RT	Area
1	Std 1	20	5.736	229775
2	Std 2	30	5.598	320992
3	Std 3	40	5.619	431129
4	Std 4	50	5.699	529137
5	Std 5	60	5.606	619839
	Avg Area			426174.4

Table 11: Analytical data for linearity-HZ **Table 12: Analytical data for linearity-TEL**



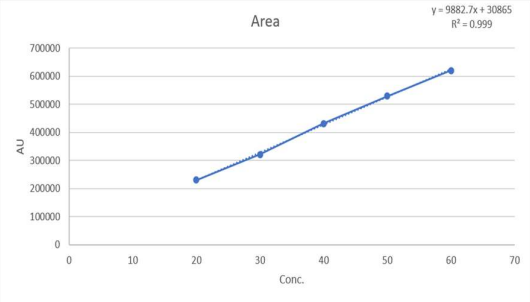


Fig 19: Linearity graph for HZ **Fig 20: Linearity graph for TEL**

Table 13: Data for calibration curve of HZ and TEL

	HZ	TEL
Parameters	**Results**	**Results**
Detection Wavelength	**282 nm**	**282 nm**
Beer's Limit	**5.05–75.75 µg/mL**	**5.05–75.75 µg/mL**
Slope	**6520.1**	**9882.7**
Intercept	**32131**	**30865**
Correleration coefficient	**0.9998**	**0.999**

Table 14: Result of detection limit

	HZ	TEL
Parameters	**Results**	**Results**
LOD	**0.79597**	**1.923344464**
LOQ	**2.41203**	**5.828316559**

DISCUSSION:

The aim of this project was to create a simple, reliable, precise, and appropriate RP-HPLC system using the Quality by Design (QbD) approach. DOE results, including ANOVA, diagnostic graphs, and model graphs, were examined for each factor. The effect of each factor on the response result was investigated in this result. In terms of analytical method creation and validation, the results of all system suitability parameters were appropriate within the limits specified by applying ICH (Q2 R1) guidelines, indicating that the system is functioning properly and can provide accurate and precise results. The established method's analysis results were validated in terms of linearity, accuracy, precision, as well as the detection and quantification limits. The developed method has many advantages, Including reproducibility of findings, rapid interpretation, easy sample preparation, and improved selectivity and sensitivity. The developed method can be used for routine research in the pharmaceutical industry for the bulk drug HZ and TEL as well as the pharmaceutical dosage type since it is stable and reproducible and takes less time.

CONCLUSION:

According to the above experimental results, this newly developed method for estimating HZ and TEL was found to be simple, precise, and accurate, with a shorter retention time that makes it more acceptable and cost effective, and it can be effectively applied for routine analysis in research institutions, quality control departments in industries, and approved testing laboratories.

FUTURE SCOPE:

- ✓ Developed RP-HPLC technology utilized in pharmaceutical business to assess tablet medication content.
- ✓ TEL and HZ bioanalytical technique development and validation in human plasma byRP-HPLC, LC-MS/MS.
- ✓ QbD can be used to develop and validate bioanalytical methods for TEL and HZ by RP-HPLC, LC-MS/MS.

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