Ind. J. Pharm. Edu. Res., 2025; 59(1s):s300-s311.
https://www.ijper.org

Original Article

Method Development for the Simultaneous Estimation of Prazosin and Polythiazide by UPLC Using QbD Approach

Pydi Geetha Bhavani[1], Durairaj Akila Devi[2,*]

[1]Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu, INDIA.
[2]Department of Pharmaceutics, School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu, INDIA.

ABSTRACT

Introduction: Prazosin is a quinazoline derivative and a selective antagonist of the α1 adrenergic receptor. The atomic structure is $C_{19}H_{21}N_5O_4$ and its molecular weight is 383.41 Prazosin is a helpful medication for managing and treating various illnesses. Through the thiazide-sensitive Na-Cl co-transporter, at the early distal tubule the diuretic polythiazide prevents active chloride re-absorption, increasing the excretion of water, salt and chloride. Its molecular formula is $C_{11}H_{13}ClF_3N_3O_4S_3$. **Materials and Methods:** The chromatographic settings were optimized using Design Expert Software. Column used is DIKMA spursil Column C18 (2.1x50 mm; 3.0 micrometre), ratio of the mobile phase KH_2PO_4: Methanol (45:55), pH 3 phosphate buffer. **Results:** The flow rate 0.3 mL/min. Run time: 5 min, wavelength: 265 nm, injection volume: 4 µL. For polythiazide and prazosin, the % RSD of precision was found to be 0.8 and 0.2, respectively. Polythiazide and Prazosin had accuracy of 100.15 and 100.30, linearity COR is 0.999 for both drugs, the corresponding LOD and LOQ were determined to be 2.91 and 10.04, accordingly. The percentages of Studies on acid, base, peroxide, thermal and photodegradation revealed that 0.27, 3.93, 10.66, 7.36 and 7.07. **Conclusion:** The UPLC-QbD validated method is used to determine the amounts of Polythiazide and Prazosin. This approach assessed the system's suitability, specificity, sensitivity, accuracy, linearity, precision and robustness according to ICH standards.

Keywords: Polythiazide, Prazosin, International Conference Harmonization, UPLC, Quality by Design.

Correspondence
Dr. Durairaj Akila Devi
Associate Professor, Department of Pharmaceutics, School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu, INDIA.
Email: cheriyanpuri@gmail.com,

Received: 03-06-2024;
Revised: 14-08-2024;
Accepted: 18-12-2024.

INTRODUCTION

Prazosin (Figure 1) is a quinazoline derivative and a selective antagonist of the α1 adrenergic receptor. 2-[4-(furan-2-carbonyl) piperazine-1-yl] is its IUPAC name. The atomic structure is $C_{19}H_{21}N_5O_4$ and its molecular weight is 383.41. Prazosin, a fused pyrimidine, is approved to treat heart failure and hypertension. From 2-amino-4,5-dimethoxy benzoic acid, 1-(4-furoyl)-4-(2-amino-6,7-dimethoxy-2-quinazolinyl)-piperazine is made. Prazosin facilitates managing and treating various illnesses, such as anxiety, refractory pulmonary edema and panic disorders. Postsynaptic α1-blockers block the pressor, vasoconstricting effects of adrenaline and noradrenaline by acting on alpha-receptive areas of vascular smooth muscle. They have a direct relaxing impact on the smooth musculature, which causes the arteries in the periphery to swell, lowering blood pressure as a result. It causes peripheral arteries, including veins and arterioles, to dilate without raising heart rate or significantly affecting sympathetic function. In those suffering from asthma or chronic obstructive pulmonary disease, prazosin has no detrimental effects on lung function. Treatment with prazosin is available for Benign Prostatic Hypertrophy (BPH), nightmares connected to Post-Traumatic Stress Disorder (PTSD), Raynaud's phenomenon and pheochromocytoma.[1-3]

By blocking the Thiazide-Sensitive Na-Cl co-Transporter (TSC) at the early distal tubule, the diuretic polythiazide increases the excretion of water, salt and chloride. Additionally, it binds to the sodium chloride transporter that is sensitive to thiazides, preventing sodium ions from passing through the renal tubular epithelium. enhances potassium excretion via the sodium-potassium exchange pathway. Blood pressure may be lowered by polythiazide due to its effects on the similarly located large-conductance calcium-activated potassium channel or its capacity to block carbonic anhydrases in smooth muscle. 6-chloro-2 methyl 3(2,2,2-trifluoro ethyl) thio] methyl is chemically speaking1-2-4 benzothiadiazide 7 sulphonamide,

DOI: 10.5530/ijper.20250020

or 3,4-dihydro-2H Polythiazide (Figure 2) is also known as 1,1-dioxide. It has the chemical formula $C_{11}H_{13}ClF_3N_3O_4S_3$.[4-6]

Analytical techniques such as UV-visible spectrophotometric, HPLC, LC-MS and RP-HPLC have been described for the measurement of Polythiazide and Prazosin combinations, according to a review of the literature. The goal of the current work was to create a novel, verified UPLC using a QbD technique for the estimation of polythiazide and prazosin simultaneously in accordance with ICH recommendations.

MATERIALS AND METHODS

Instruments

Instruments are UPLC waters Acquity model, 2996 PDA detector with Empower-3, Ultra Violet/Visible spectrophotometer (LABINDIA UV3000+), Adwa-AD1020 pH meter, beakers, burettes, pipettes and weighing machines are employed.

Chemicals Used

Polythiazide and Prazosin drugs from Honour Lab, KH_2PO_4, Water, Methanol, Acetonitrile and Ortho phosphoric Acid.

Methodology

It has factor coding. Type III: Partial sum of squares is the result. Noise could be the likely source of a large F-value, as indicated by the Model F-value of 2.91 and the 8.62% likelihood. *p*-values below 0.0500 suggest that the model terms are significant Here, BC is an important model term. A value greater than 0.1000 renders the model terms meaningless. If your model has a large number of extraneous terms (beyond those required to maintain hierarchy), model reduction may help. When compared to the pure error, the 2.26 Lack of Fit F-value indicates that the Lack of Fit is not statistically significant. There is a 22.34% chance that noise is the reason for a significant (Tables 1-6). Lack of Fit F-value (Figure 3).

UPLC Method Development

Mobile Phase Optimization

Initially, several pH combinations were tested along with varying concentrations of methanol, phosphate buffer, orthophosphoric acid buffer and acetonitrile: methanols were investigated as mobile phases. In the end, the pH of Phosphate Buffer 3 and methanol in the mobile phase were adjusted to 45:55 v/v, respectively.

Wavelength selection

To get the UV spectrum, the concentrations of 10 µg/mL Polythiazide and 10 µg/mL Prazosin in diluents (mobile phase composition) were scanned in the 200 nm-400 nm range. The wavelength from the UV spectrum that was selected was 265 nm. Both medications exhibit high absorption at this wavelength.

Optimized Chromatographic Conditions

The ideal chromatographic conditions are as follows: KH_2PO_4: Methanol (45:55) mobile phase volume, a 2.1 mmx50 mm, 3.0 mm DIKMA spursil C18 column, an isocratic mode of separation, 25°C ambient temperature, a 0.3 mL per min flow rate, an observed wavelength of 265 nm, an injection volume of 4 µL and a 5 min run time.

Preparation of Phosphate Buffer 3 pH

Potassium dihydrogen orthophosphate (0.1 M) in an equivalent volume should be added to a 1000 mL volumetric flask. Once the flask has been filled with HPLC water, vacuum-filter the mixture through a 0.45 µ filter, degas in an ultrasonic water bath for 10 min and use OPA to get the pH down to-3.

Mobile Phase Preparation

Phosphate buffer (450 mL; 45%) and methanol (550 mL; 55%) were combined in precisely measured volumes, degassed for 10 min in water bath and then vacuum filtered through a 0.45 µ filter.

Preparation of the Diluent

The diluent was the Mobile Phase.[7-9]

Standard Solution Preparation

Precisely measure out and add 25 mg of working standard prazosin and 5 mg of polythiazide in 100 mL volumetric flask. Utilizing the solvent, increase the volume to the required amount after adding roughly 70 mL of Diluent and sonicating to fully dissolve the components. (Sorted according to inventory) (Table 7). Fill a 10 mL volumetric flask with 1 mL of the previously mentioned stock solutions and then dilute it with diluent to the appropriate level (Figure 4).

Sample Solution Preparation

Weigh out precisely 5 mg of polythiazide and 25 mg of prazosin into a 100 mL dry volumetric flask. Subsequently, sonicate the sample until it dissolves completely, then top out the flask with enough solvent to bring the volume up to the required level after adding about 70 mL of diluent. (Stock resolution) In 10 mL volumetric flask with 1 mL (1.0 mL) of the aforementioned stock solutions using a pipette and then dilute with diluent to the appropriate concentration (Table 8).

Procedure

Measure the regions for the polythiazide and prazosin peaks after adding 4 µL of the standard to the chromatographic device. Next, use the formulas to calculate the assay percentage.

System Suitability

In the usual solution, the tailing factor of the prazosin and polythiazide peaks shouldn't be greater than 2.0. Theoretical

Table 1: Build Information.

Sl.No run time	Factor 1 Buffer pH	Factor 2 Flow rate	Factor 3 Column Temp	Response 1 Retention Time of Prazosin (min)	Response 2 Plate Count of Prazosin
1	5	0.25	25	1.542	3760
2	4	0.3	35	1.448	3650
3	4	0.3	25	1.523	3550
4	5	0.3	30	1.446	3647
5	3	0.3	30	1.458	3620
6	4	0.25	30	1.52	3647
7	4	0.2	25	1.496	3600
8	5	0.25	35	1.497	3523
9	4	0.25	30	1.478	3547
10	4	0.2	35	1.52	3600
11	5	0.2	30	1.448	3587
12	4	0.25	30	1.498	3692
13	4	0.25	30	1.488	3587
14	3	0.25	35	1.51	3650
15	3	0.2	30	1.52	3654
16	3	0.25	25	1.496	3687
17	4	0.25	30	1.485	3600

Table 2: Retention Time of Prazosin.

Origin	In Order	Lack of Fit	Adjusted	Predicted	
linear	0.4050	0.0924	0.0086	-0.6103	
2FI	0.1664	0.1195	0.2066	-1.2322	
Quadratic	0.0950	0.2234	0.5185	-1.2423	Suggested
Cubic	0.2234		0.6875		Aliased

Table 3: ANOVA for quadratic model.

Origin	Composition of Squares	DF	Mean Square	Lack of Fit	*p*-Value	
Model	0.0108	9	0.0012	2.91	0.0862	
Buffer pH-A	0.0003	1	0.0003	0.7927	0.4028	
Flow Rate-B	0.0015	1	0.0015	3.62	0.0988	
Column Temp-C	0.0008	1	0.0008	2.05	0.1954	
Buffer pH, Flow Rate	0.0009	1	0.0009	2.19	0.1821	
Buffer pH, Column Temp	0.0009	1	0.0009	2.12	0.1886	
Flow Rate, Column Temp	0.0025	1	0.0025	5.97	0.0445	
$(\text{Buffer pH})^2$	0.0001	1	0.0001	0.3277	0.5849	
$(\text{Flow Rate})^2$	0.0017	1	0.0017	4.17	0.0805	
$(\text{Column Temp})^2$	0.0022	1	0.0022	5.48	0.0518	
Residual value	0.0029	7	0.0004			
F-Value	0.0018	3	0.0006	2.26	0.2234	Significant
Error	0.0011	4	0.0003			
Correlation	0.0136	16				

plates of at least 2000 should be present for the polythiazide and prazosin peaks in standard solution. The standard solution's polythiazide and prazosin peaks need to have a resolution of two or higher.

Calculation: (Polythiazide)

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Averageweight}{LabelClaim} * \frac{P}{100} * 100$$

In this case, AT stands for average area counts during sample processing, AS-standard preparation's average area counts, WS-Working standard weight, in mg, P- Working standard percentage Purity, Label Claim (LC)=micro gram /mL.

System Adequacy Outcomes

The typical injection has a tailing factor of 1.46. Plates 4725.92 obtained theoretically using traditional injection.

Assay Results: (Polythiazide)

$$\frac{14685}{14633} * \frac{5}{100} * \frac{1}{10} * \frac{100}{164.5} * \frac{10}{1} * \frac{164.5}{5} * \frac{99.8}{100} * 100 = 100.15$$

Calculation: (Prazosin % Assay)

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Averageweight}{LabelClaim} * \frac{P}{100} * 100$$

Where AT is the sample preparation avg area counts, AS=standard preparation avg area counts, WS=Working std weight, measured in mg, P=Working standard purity percentage, Label Claim (LC)=mg/mL.

System Suitability Outcome

1. A tailing factor 1.29 is obtained with the standard injection
2. Hypothetical Plates: 6256.39 derived using the conventional injection.

3.18 is the resolution attained with the usual injection.

Assay Results: (For Prazosin)

$$\frac{96652}{96256} * \frac{25}{100} * \frac{1}{10} * \frac{100}{164.5} * \frac{10}{1.} * \frac{164.5}{25} * \frac{99.8}{100} * 100 = 100.30\%$$

RESULTS

Method Validation Summary, Precision

The stock solution is prepared as follows

After carefully weighing, fill a 100 mL dry volumetric flask with 25 mg of the working standard prazosin and 5 mg of polythiazide. Add around 70 mL of the dissolved diluent and use the same solvent to bring the volume up to the required level. (Assembly of stocks) Place 1 mL of the previously mentioned stock solutions into a 10 mL volumetric flask and dilute it to the desired concentration using diluent (Table 9).

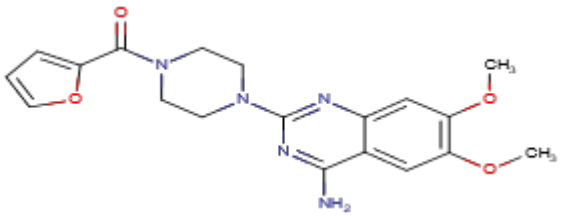

Figure 1: Structure of Prazosin.

Table 4: Fit Summary.

Source	Sequential value	Lack of Fit value	Adjusted R^2	Predicted R^2	
Mean	Less than 0.0001				Suggested
Linear	0.7428	0.4049	-0.1224	-0.6872	
2FI	0.3183	0.4228	-0.0425	-1.4256	
Quadratic	0.6867	0.2764	-0.2212	-4.3318	
Cubic	0.2764		0.1088		Aliased

Table 5: Sequential Model Sum of Squares.

Source	Sum of Squares	d_f	Mean Square	F-value	*p*-value	
Mean vs Total	2.232E+08	1	2.232E+08			
Linear vs Mean	4973.50	3	1657.83	0.4184	0.7428	
2FI vs Linear	14709.00	3	4903.00	1.33	0.3183	
Quadratic vs 2FI	6622.92	3	2207.64	0.5121	0.6867	
Cubic vs Quadratic	17593.50	3	5864.50	1.86	0.2764	Aliased
Residual	12585.20	4	3146.30			
Total	2.233E+08	17	1.313E+07			

Method

Area was measured after the standard solution was administered 6 times. injections of UPLC. The percentage RSD for the region of 6 replicate injections was found within the necessary bounds.

Acceptance Criteria

For the outcomes of the six standard injections, the percentage RSD shouldn't be more than 2% (Table 10).

Intermediate Precision

The method ruggedness, or intermediate precision, precision was conducted on a different day.

Stock solution preparation

Accurately weigh and transfer 5 mg of polythiazide and 25 mg of the working standard prazosin into a 100 mL dry volumetric flask. After fully dissolving the contents with sonication, add around 70 mL of diluent, then utilize the same solvent to bring the volume up to the required level. (Sorted according to inventory). To a 10 mL volumetric flask, add 1 mL of the previously listed stock solutions. Then, dilute with diluent to the necessary level.

Procedure

Six injections of the carefully prepared standard solutions were made the other day and UPLC was used to measure the area of each injection. It was discovered that the %RSD for the six replicate injections fell between the required ranges. Outcomes for the areas of polythiazide and prazosin are outlined (Table 11).

Acceptance Criteria

For the outcomes of the six standard injections, the percentage RSD shouldn't be more than 2%.

Specificity

To increase specificity, Standard and Blank are introduced into the system. Peak interference in the blank has no effect on retention time of analytical peaks.

Accuracy

Standard stock solution Preparation

Precisely measure out and add 25 mg of working standard prazosin and 5 mg of polythiazide to a 100 mL dry volumetric

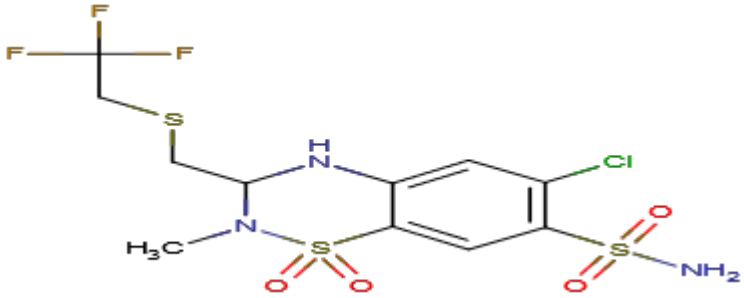

Figure 2: Structure of Polythiazide.

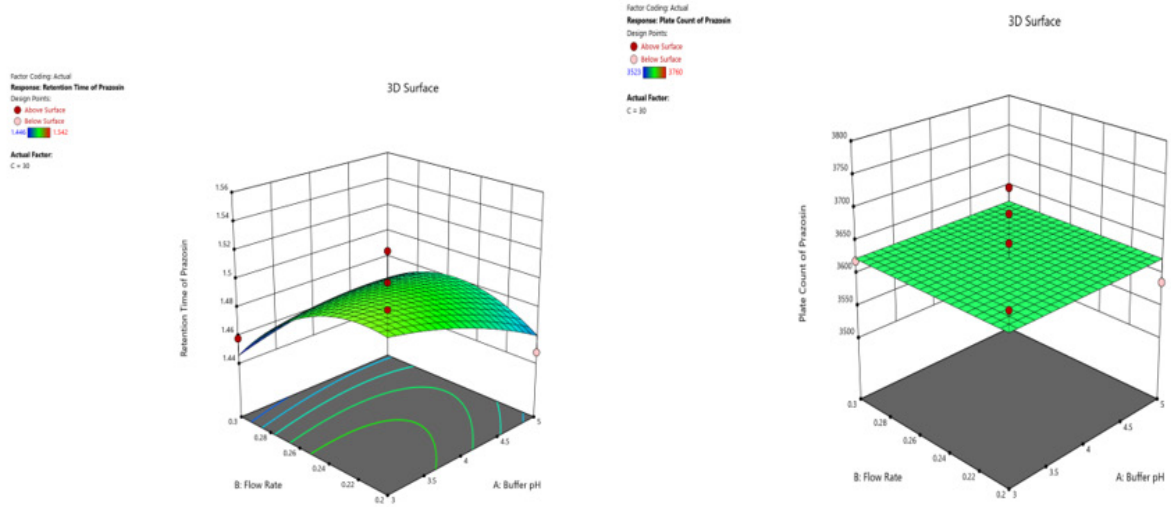

Figure 3: Retention Time and Plate Count of Prazosin.

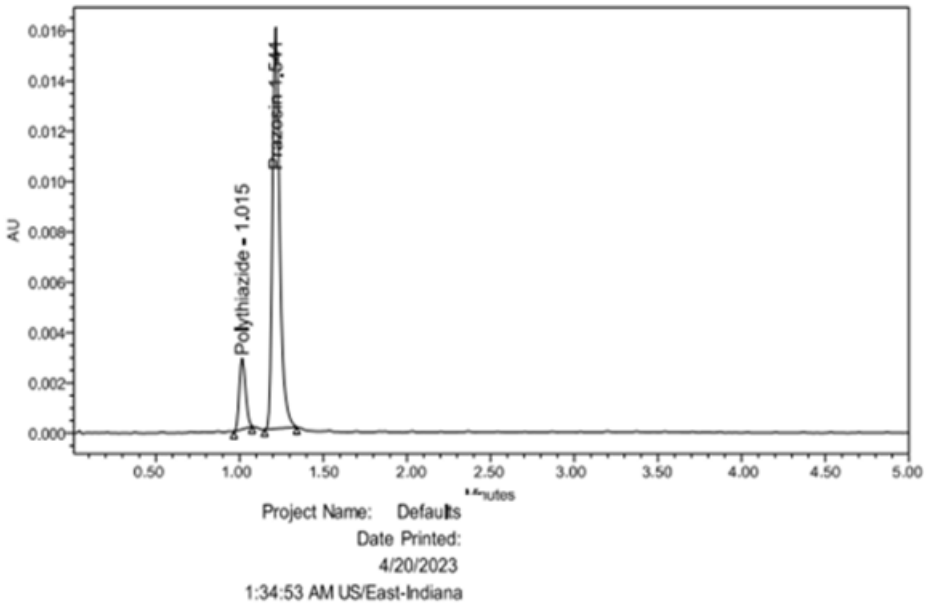

Figure 4: Standard Chromatogram.

Table 6: Model Summary Statistics.

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	62.95	0.0881	-0.1224	-0.6872	95298.79	
2FI	60.66	0.3485	-0.0425	-1.4256	1.370E+05	
Quadratic	65.66	0.4657	-0.2212	-4.3318	3.012E+05	
Cubic	56.09	0.7772	0.1088		*	Aliased

flask. The mixture should then be completely dissolved using a sonicated after adding about 70 mL of diluent. Continue adding solvent to the flask until the desired volume is reached (Ordered by stock). Moreover, fill a 10 mL volumetric flask with 1 mL of the previously listed stock solutions using a pipette and then dilute with diluent to the appropriate concentration.

Preparation Sample solution

Preparation of 50% solution

In a 100 mL dry volumetric flask, gently transfer 2.5 mg of polythiazide and 12.5 mg of working standard prazosin. To fully dissolve the mixture, sonicate it after adding around 70 mL of diluent. Then, add more solvent until the volume reaches the desired level. (Sorted according to inventory). Additionally, pipette 1 mL of the stock solutions that were previously mentioned into a 10 mL volumetric flask. Dilute the mixture to the appropriate concentration using diluent after that.

Preparation of 100% solution

After carefully weighing, put 25 mg of prazosin, the working standard and 5 mg of polythiazide into a 100 mL dry volumetric flask. After that, add about 70 mL of diluent, sonicate to completely dissolve everything and then use the same solvent to raise the volume to the necessary level. (Assembly of stocks) Moreover, pipette 1 mL of the previously indicated stock solutions into a 10 mL volumetric flask and then dilute with diluent to the proper concentration.

Preparation of 150% solution

Precisely measure out 7.5 mg of polythiazide and 37.5 mg of prazosin working standard, then transfer them into a 100 mL dry volumetric flask. Then add about 70 mL of Diluent, sonicate to dissolve it all and then use the same solvent to bring the volume up to the required level. (Compositing stocks) Additionally, pipette 1mL of the stock solutions that were previously mentioned into a 10 mL volumetric flask. Dilute the mixture to the appropriate concentration using diluent after that.

Procedure

Should inject the standard solution together with accuracy solutions of 50%, 100% and 150%. Calculate the mean and individual recovery values for polythiazide and prazosin, as well as the amounts added and discovered.

Acceptance Criteria

Between 98.0 and 102.0% should be the recovery percentage.

Linearity

Preparation of stock solution

Place 5 mg of polythiazide and 25 mg of prazosin, the working standard, into a 100 mL dry volumetric flask after carefully weighing. Subsequently, incorporate approximately 70 mL's of diluent, sonicate to fully dissolve all components and utilize the same solvent to increase the volume to the required amount.

Preparation of Level I

Pour 0.25 mL of the previously indicated stock solutions into a 10 mL volumetric flask and then dilute with diluent to the appropriate concentration.

Preparation of Level II

To get the right concentration, dilute 10 mL of the volumetric flask holding 0.5 mL of the previously indicated stock solutions with diluent.

Preparation of Level III

Use diluent to dilute 0.75 mL of the previously listed stock solutions to the required concentration in a 10 mL volumetric flask.

Preparation of Level IV

To obtain the appropriate dilution, diluent has been added to a volumetric flask holding 1.0 mL of the previously indicated stock solutions.

Preparation of Level V

1.25 mL of the previously mentioned stock solutions were added to 10 mL volumetric flasks and they were then suitably diluted with diluent.

Preparation of Level VI

A 10 mL volumetric flask has been filled with 1.5 mL of the previously indicated stock solutions; use diluent to dilute to the appropriate amount.

Procedure

Measure the peak area after each level has been introduced into the chromatographic apparatus. Plot the peak area vs. concentration on the X- and Y-axes to find the correlation coefficient.

Acceptance Criteria

A minimum correlation coefficient of 0.999 is necessary.

Limit of Detection: (for Polythiazide)

Preparation of 0.23 µg/mL solution

Accurately measure the working standard polythiazide and pour it into a 100 mL dry volumetric flask with 5 mg of the medication. Once about 70 mL of diluent has been added and thoroughly

Table 7: Standard Values of Polythiazide and Prazosin.

Peak Name: Polythiazide.

	Peak Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Polythiazide	1.012	14601	2825	1.16	3083.51
2	Polythiazide	1.015	14715	2847	1.13	3479.74
3	Polythiazide	1.043	14585	2822	1.13	3260.44
Mean			14633.7			
Std.Dev			70.9			
%RSD			0.5			

Peak Name: Prazosin.

	Peak Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Prazosin	1.529	96100	15802	1.31	3760.10
2	Prazosin	1.535	97019	15953	1.37	3205.44
3	Prazosin	1.541	95651	15728	1.33	3815.62
Mean			96256.7			
Std.Dev			697.3			
%RSD			0.7			

Table 8: Sample Values of Polythiazide and Prazosin.

Peak Name: Polythiazide.

	Peak Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Polythiazide	2.044	14615	2828	1.16	3178.51
2	Polythiazide	2.047	14685	2841	1.13	3234.04
3	Polythiazide	2.049	14756	2855	1.13	3456.44
Mean			14685.3			
Std.Dev			49.5			
%RSD			0.3			

Peak Name: Prazosin.

	Peak Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Prazosin	1.533	97014	15952	1.31	3700.10
2	Prazosin	1.535	96315	15837	1.37	3765.44
3	Prazosin	1.542	96627	15889	1.33	3321.62
Mean			96652.0			
Std.Dev			494.3			
%RSD			0.5			

Table 9: % RSD of Precision for Polythiazide and Prazosin.

Injection	Area for Polythiazide	Area for Prazosin
1 Injection	14572	96756
2 Injection	14497	96245
3 Injection	14756	96786
4 Injection	14678	96458
5 Injection	14565	96452
6 Injection	14767	96753
Avg	14639.2	96575.0
Standard Deviation	111.1	222.1
Percentage Relative Standard Deviation	0.8	0.2

dissolved by sonicating, the volume should be increased using the same solvent. (How the stocks are arranged). 1mL of the previously mentioned stock solutions should be pipetted into a 10 mL volumetric flask and diluted to the appropriate concentration with diluent. Additionally, pipette 0.46 mL of the stock solution that was previously described into a 10 mL volumetric flask and dilute to the required concentration using diluent.

Calculation of S/N Ratio

Average Baseline Noise measured from Blank at 45 µV. The LOD solution yielded a signal of 131 µV. S/N is 2.91, or 131/45.

Acceptance Criteria

The S/N Ratio value for the LOD solution should be 3.

Limit of Quantification

Preparation of 0.80 µg/mL solution

Weigh out the working standard polythiazide precisely and add 5 mg to a 100 mL dry volumetric flask. Next, add roughly 70 mL of diluent, sonicate to dissolve it fully and then increase the amount to the required level using the same solvent. (Stock arrangement). Fill a 10 mL volumetric flask with 1mL of the aforementioned stock solutions via pipette and then dilute with diluent to the appropriate concentration. Pipette 1.6 mL of the previously mentioned stock solution into a 10 mL volumetric flask. Use diluent to dilute the stock solution to the required volume.

Calculation of S/N Ratio

The mean baseline noise, calculated at 45 µV from the blank signal S/N equals 452/45, or 10.04, when measured at 452 µV from the LOQ solution.

Acceptance Criteria

For the LOQ solution, the S/N Ratio needs to be 10.

Limit of Detection: (for Prazosin): Making a solution with 0.21 µg/mL

In a 100 mL dry volumetric flask, add precisely 25 mg of the prazosin working standard. Once the substance is completely dissolved, add around 70 mL of diluent and sonicate the flask again.

Repeat until the desired volume is reached. (The configuration of stocks). A 10 mL volumetric flask should also be pipetted with 1 mL of the previously indicated stock solutions and the volume should be diluted with diluent to the proper concentration. The previously mentioned stock solution should be pipetted into a 10 mL volumetric flask in an amount of 0.084 mL. Use diluent to dilute the stock solution to the appropriate concentration.

Calculation of S/N Ratio

At 45 µV, the mean baseline noise from Blank LOD solution yielded a signal of 133 µV. S/N is equivalent to 2.96, or 133/45.

Acceptance Criteria

For the LOD solution, the S/N Ratio value should be 3.

Limit of Quantification: Preparation of 0.72 µg/mL solution

Transfer 30 mg of the prazosin working standard precisely measured into a 100 mL dry volumetric flask. After that, fill the flask to the required volume with additional solvent by adding around 70 mL of diluent, sonicating to completely dissolve the substance. (Arrangement of stocks). After pipetting 1.5 mL of the previously specified stock solutions into a 10 mL volumetric flask, dilute it to the necessary amount with diluent Once the necessary volume is attained, dilute the stock solution with diluent after pipetting 0.287 mL of it into a 10 mL volumetric flask.

Calculation of S/N Ratio

Avg Baseline Noise measured at 45 µV from Blank Signal Measured at 454 µV from the LOQ solution S/N is equal to 454/45, or 10.09.

Acceptance Criteria

For the LOQ solution, the S/N Ratio ratios need to be 10.

Procedure for LOD and LOQ

The LOD and LOQ solutions were injected three times and following each injection, the area was measured using UPLC. It was discovered that the %RSD for the six replicate injections fell between the required ranges.

Table 10: Precision Values of Polythiazide and Prazosin.
Peak Name: Polythiazide.

	Peak Name	RT	Area	Height	USP Tailing	USP Plate count
1	Polythiazide	2.012	14572	2819	1.16	3987.51
2	Polythiazide	2.015	14497	2805	1.16	3754.63
3	Polythiazide	2.017	14756	2855	1.13	3357.74
4	Polythiazide	2.029	14678	2840	1.14	3889.31
5	Polythiazide	2.037	14565	2818	1.13	3009.44
6	Polythiazide	2.041	14767	2857	1.14	3137.26
Mean			14639.2			
Std.Dev			111.1			
% RSD			0.8			

Peak Name: Prazosin.

	Peak Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate count
1	Prazosin	2.533	96756	15910	2.65	1.31	3700.10
2	Prazosin	2.541	96245	15826	2.65	1.32	3564.26
3	Prazosin	2.543	96786	15915	2.66	1.37	3733.44
4	Prazosin	2.552	96458	15861	2.65	1.38	3220.72
5	Prazosin	2.555	96452	15860	2.64	1.33	3092.62
6	Prazosin	2.557	96753	15909	2.63	1.33	3282.62
Mean			96575.0				
Std.Dev			222.1				
% RSD			0.2				

Robustness

In order to assess the effect on the process, intentional modifications were made to the Temperature Variation, Flow rate and Mobile Phase composition as part of the Robustness. The range of the flow rate was 0.27 to 0.33 mL/min. The analysis employed various flow rates and method flow rates to synthesis 25 ppm of prazosin and 5 ppm of polythiazide. The method was significantly impacted by the flow rate variation, it can be concluded from the analysis of the previously provided data. Thus, it implies that even in the case of a ±10% variation in the flow rate, the method stays constant. The Wavelength's Organic makeup fluctuated between ±10%. In addition to the modified mobile phase composition, the method's actual mobile phase composition was also used to create and analyse 25 ppm of prazosin and 5 ppm of polythiazide. By examining the previously provided information, 10% of the variation can be found. The organic content of the mobile phase significantly altered the strategy. Consequently, it demonstrates that the strategy is adaptable to modifications in the mobile phase ±10.

Table 11: % RSD of Intermediate Precision for Polythiazide and Prazosin.

Injection	Area for Polythiazide	Area for Prazosin
1 Injection	14872	96836
2 Injection	14756	96486
3 Injection	14582	96435
4 Injection	14643	96856
5 Injection	14869	96456
6 Injection	14668	96786
Avg	14731.7	96642.5
Standard Deviation	121.2	203.0
Percentage Relative Standard Deviation	0.8	0.2

DEGRADATION STUDIES

Preparation of stock: Sample Solution Preparation

Precisely measure out and incorporate 5 mg of Polythiazide and 25 mg of powdered Prazosin tablet into a 100 mL dry volumetric flask. Once the contents have been thoroughly dissolved by sonicating the flask with approximately 70 mL of diluent, top it off with additional solvent to get the volume up to the required level. (Compositing stocks).

Hydrolytic degradation under acidic condition

Transfer 1 mL of the previously mentioned solution using a pipette into a 10 mL volumetric flask. Then, add 3 mL of 0.1N HCl. After being stored at 60°C for 24 hr, the volumetric flask was filled to the brim with diluent and neutralized with 0.1 N NaOH. Syringe filters with a 0.44 micron pore size are used to transfer the solution into vials.

Hydrolytic degradation under alkaline condition

The above solution is taken into a 10 mL vol flask in volume of 1 mL. Add 3 mL of 0.1N NaOH after that. Volumetric flask was filled to 10 mL with diluent and neutralized with 0.1N HCl following a 24 hr incubation period at 60°C. Transfer the solution into vials using syringe filters with a 0.44 micron pore size.

Thermal induced degradation

In a petridish, a sample of powdered prazosin and polythiazide tablets was roasted in a hot air oven for 3 hr at 110°C. Subsequently, the material underwent extraction, dilution with diluents and UPLC analysis.

Oxidative degradation

Pipette 10 mL volumetric flask holding 1 mL of the previously mentioned stock solution taken 1 mL of 30% w/v H_2O_2. Diluent then used to regulate volume. After that, the volumetric flask was let to stand at room temperature for a further 15 min. Transfer the solution into vials using syringe filters that have a 0.45-micron filter.

Table 12: Percentage Degradation Results.

Sample Name	Prazosin		Polythiazide	
	Area	% Degraded	Area	% Degraded
Standard	11032553		4865223	
Acid	12162859	10.25	4878390	0.27
Base	12096345	9.64	5056646	3.93
Peroxide	11688369	5.94	5383718	10.66
Thermal	11256358	2.03	5223252	7.36
Photo	12256958	11.10	4521477	7.07

DISCUSSION

An attempt has been made for simultaneous estimation of polythiazide and prazosin by QbD approach. Optimized chromatographic conditions were finalized by selecting mobile phase ratio of potassium dihydrogen phosphate and methanol (45:55 v/v), pH at 3, DIKMA Spursil C_{18} (2.1 mmX50 mm, 3.0 µm); Run time 5min; Injection volume 4 µL. %RSD of precision for polythiazide and prazosin was found to be 0.8 and 0.2 respectively. Polythiazide and prazosin had accuracy of 100.15 and 100.30; linearity correlation is 0.99 for both drugs, the corresponding LOD and LOQ were determined to be 2.91 and 10.04 respectively. Ruggedness system suitability results of USP plate count and USP tailing factor for polythiazide is 1.46 and 4725.92 and for prazosin is 6256.39 and 1.29 respectively. All the parameters according to ICH guidelines were performed and observed within the limits. Literature survey reveals a retention time which is more than 3 min but in the developed method retention time is found to be less than 2 min which shows less retention for prazosin was 2.989. Degradation studies of acid, base, peroxide, thermal and photo degradation were 0.27, 3.93, 10.667, 7.36 and 7.07. No impurities were found and degradation of the sample was within the limits.

CONCLUSION

Quality by Design (QbD), a scientific technique, is widely employed in the pharmaceutical sector for product development. It lowers product variability and related risks. A validated UPLC-QbD method was used to determine the amounts of Polythiazide and prazosin. This approach assessed the system's suitability, specificity, sensitivity, accuracy, linearity, precision and robustness according to ICH standards. As a result, the UPLC-QbD-based method that was presented was used for routine tablet dosage form analysis and quality control.

ACKNOWLEDGEMENT

Authors are thankful to Management, HOD of Analysis Department of Vikas Institute of Pharmaceutical Sciences Rajahmundry for continuous support in my work and also thankful to Qualychrome Research Labs, Hyderabad for providing samples.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

QbD: Quality by Design; **UPLC:** Ultra Performance Liquid Chromatography; **Min:** Minutes; **%:** Percentage; **ICH:** International Conference Harmonization; **mL:** Milliliters; **ºC:** Degree Celsius; **PPM:** Parts Per Minutes; **% RSD:** Percentage Relative Standard Deviation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **µg/mL:** micro gram per milliliter; **conc:** concentration; **gms:** grams; **mg:** milligrams; **HPLC:** High Performance Liquid Chromatography; **UV:** Ultra Violet Spectroscopy; **Nm:** nanometer; **DoE:** Design of Experiments.

SUMMARY

According to ICH guidelines precision value was found to be accuracy means recovery of Polythiazide and Prazosin were 100.15 and 100.30 and the Linearity correlation coefficient was 0.999 for both drugs; LOQ and LOD of Polythiazide and Prazosin were found to be 10.04 and 2.91. The experimental design explains the interaction between the phase that is mobile and the pH value response that will be recorded. Percentage degradation studies of Acid, base, Peroxide, Thermal and Photo were found to be 0.27, 3.93, 10.66, 7.36 and 7.07 where degradation studies are less and more stability of a compound.[10-15]

REFERENCES

1. Uttam Prasad Panigrahy, K Naga Vishnu Kumari, T Ram Mohan Reddy, K Abbulu. RP-HPLC method development and validation for simultaneous estimation of Prazosin and Polythiazide in bulk and pharmaceutical dosage form. Research J. Pharm. and Tech, 2020;13(4):1779.
2. M. Mukkanti Eswarudu, A.Lakshmana Rao, K. Vijay. Bioanalytical method development and validation for simultaneous determination of Prazosin and Polythiazide drugs in spiked human plasma. IJPCBS, 2019;9(1):61-70.
3. M. Suma Pallavi, P. Prapulla. Simultaneous estimation of Prazosin and Polythiazide in bulk and fixed dose combination (tablets) by RP-HPLC. IAJPS, 2022;9(12):876-84.
4. Rama Krishna B, Supriya P, Venkata Lakshmi V and Naidu N.V.S. RP-HPLC Method development and validation for the simultaneous estimation of prazosin and polythiazide in bulk and tablet dosage forms. International journal of current research, 2018;10(3):66228-35.
5. S K Godasu, D Varun, Narmada Valla Keerthi, Mohathasim Bilah A, S.A Sreenivas. Simultaneous Estimation of Prazosin and Polythiazide in Bulk and Fixed Dose Combination (Tablets) by RP-HPLC. A Journal of Pharmaceutical Science: 2021;12(3).
6. Shabana Naz Shah. Development and validation of simple UV-Spectrophotometric method for quantification of Prazosin in API and solid dosage formulation. IJQPA: 2015;1(1):110-4.
7. Alankar Shrivastava, Vipin B. Gupta. Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Prazosin, Terazosin and Doxazosin in Pharmaceutical Formulations. Sci Pharma: 2012;80(3):619-31.
8. G. Dharmamoorthy, K. S. Nataraj, A. Krishna Manjari Pawar. New validated stability indicating Reverse Phase High Performance Liquid chromatography Method for the simultaneous estimation of Prazosin and Polythiazide in their formulations in Human Plasma. Asian Journal of Pharmaceutics: 2020:14(2):252-64.
9. Alankar Shrivastava, Vipin Bihari Gupta. Simultaneous determination of two alpha-one Adrenoreceptor blockers Terazocin and Prazocin using Tamsulosin as internal standard. International Journal of Pharmacy and Pharmaceutical sciences: 2012;4(3).
10. Biljana Panzova, Miroslava Ilievska, Gordana Trendovska, Bogdan Bogdanov. Simultaneous determination of polythiazide and prazosin in tablets by second-order derivative UV spectroscopy. International Journal of Pharmaceutics: 1991:187-90.
11. Veera Raghava Raju Thummala, Raja Kumar Seshadri, Satya Sankarsana Jagan Mohan Tharlapu, Mrutyunjaya Rao Ivaturi, Someswara Rao Nittala. Development and Validation of a UPLC Method by the QbD-Approach for the Estimation of Rabeprazole and Levosulpiride from Capsules. Sci. pharma: 2014;82(2):307-26.

12. L Kothapalli, P. Darekar, A. Thomas Quality by Design (Qbd) approach for method development for Azilsartan Medoxomil using UPLC: application to hydrolytic, thermal and oxidative degradation kinetics. IJPSR: 2020;11(8):3867-75.
13. Sowmya HG, M. Gnana Ruba Priya, V. Murugan. Implementation of Quality by Design Approach for Method Development and Validation. RJPT: 2021;15(1):436-40.
14. Hanimi Reddy Bapatu, Ravi Kumar Maram, Woo Hyung Cho, Venkat Bhaskar Rao Pasagadugula. QbD Approach Method Development for Estimation of Dabigatran Etexilate along with Its Impurities and Identification of Degradants in Capsule Dosage Form. American Journal of Analytical Chemistry: 2016;7(6).
15. CV Chandarana, NR Mithani, PR Prajapat. Analytical Quality by design: A Review for chromatography. Biomedical Journal of Science and Technical Research: 2022;42(3):33714-32.

Cite this article: Bhavani PG, Devi DA. Method Development for the Simultaneous Estimation of Prazosin and Polythiazide by UPLC Using QbD Approach. Indian J of Pharmaceutical Education and Research. 2025;59(1s):s300-s311.