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RESEARCH ARTICLE

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF PRAZOCIN AND POLYTHIAZIDE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Prazocin and Polythiazide in Tablet dosage form. Chromatogram was run through Std Discovery C18 150 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.1%OPA: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% OPA buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 270.0 nm. Retention time of Prazocin and Polythiazide were found to be 2.316 min and 3.176. %RSD of the Prazocin and Polythiazide were found to be 0.5 and 0.7 respectively. %Recovery was obtained as 100.49% and 100.40% for Prazocin and Polythiazide respectively. LOD, LOQ values obtained from regression equations of Prazocin and Polythiazide were 0.079, 0.240 and 0.03, 0.08 respectively. Regression equation of Prazocin is y = 50050.x + 7773 and of Polythiazide was y = 95434.x + 6175. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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INTRODUCTION

Prazosin is a selective α-$_1$-adrenergic receptor antagonist used to treat hypertension. It has also been used to decrease urinary obstruction and relieve symptoms associated with symptomatic benign prostatic hyperplasia. α_1-Receptors mediate contraction and hypertrophic growth of smooth muscle cells. Antagonism of these receptors leads to smooth muscle relaxation in the peripheral vasculature and prostate gland. Prazosin has also been used in conjunction with cardiac glycosides and diuretics in the management of severe congestive heart failure. It has also been used alone or in combination with β-blockers in the preoperative management of signs and symptoms of pheochromocytoma. A thiazide diuretic with actions and uses similar to those of hydrochlorothiazide. (From Martindale, The Extra Pharmacopoeia, 30th ed, p826)

MATERIALS AND METHODS

Chemicals and Reagents

Prazocin and Polythiazide pure drugs (API), Combination Prazocin and Polythiazide tablets (MINIZIDE 2), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

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Instruments and Chromatographic Conditions

Electronics Balance-Denver**,** p^H meter-BVK enterprises, India**,** Ultrasonicator-BVK enterprises**,** WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Prazocin and Polythiazide solutions. The mobile phase used was 0.1% Ortho phosphoric: Acetonitrile in the ratio of 60:40 run Discovary 150 x 4.6 mm, 5µ, column at a rate of 1 ml/min. for 6 min at Temperature 30°C and Optimized wavelength was 270nm at the injection volume of 10µL.

Preparation of Solvents and Solution

Diluent

Based up on the solubility of the drugs, diluent was selected. Acetonitrile and Water taken in the ratio of 50:50.

Preparation of buffer

Preparation of 0.1% Ortho phosphoric acid Buffer

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Preparation of Mobile Phase

Mobile phase was prepared my mixing 0.1% Ortho phosphoric: Acetonitrile in the ratio of 60:40 and sonicated using ultrasonic bath to degas and subjected to vacuum filtration with 0.45µ Millipore Nylon filter.

Preparation of Standard stock solutions

Accurately weighed 10mg of Prazocin, 1mg of Polythiazide and transferred to 10ml and 10ml individual volumetric flasks and 3/4 th of diluents was added to this flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (400µg/ml of Prazocin and 100µg/ml Polythiazide)

Preparation of Standard working solutions (100% solution)

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (40µg/ml of Prazocin and 10µg/ml of Polythiazide)

Preparation of Sample stock solutions

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (400µg/ml of Prazocin and 10µg/ml of Polythiazide).

Method Validation

As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

Specificity

It is the ability of analytical method to measure the response of the analyte and have no interference from other extraneous components and well resolved peaks are obtained.

Linearity

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (10µg/ml of Prazocin and 2.5µg/ml of Polythiazide).

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (20µg/ml of Prazocin and 5µg/ml of Polythiazide).

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (30µg/ml of Prazocin and 7.5µg/ml of Polythiazide).

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. 40µg/ml of Prazocin and 10µg/ml of Polythiazide).

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (50µg/ml of Prazocin and 12.5µg/ml of Polythiazide).

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (60µg/ml of Prazocin and 15µg/ml of Polythiazide).

Accuracy

Preparation of Standard stock solutions: Accurately weighed 10mg of Prazocin, 1mg of Polythiazide and transferred to 10ml and 10ml individual volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labelled as Standard stock solution. (400µg/ml of Prazocin and 100µg/ml Polythiazide).

Preparation of 50% Spiked: 5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria

The % Recovery for each level should be between 98.0 to 102

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Prazocin, Polythiazide, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Prazocin, Polythiazide, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

System Suitability: The system suitability parameters were determined by preparing standard solutions of Prazocin (100ppm) and Polythiazide (25ppm) and the solutions were injected six times and the parameters like peak tailing,

resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

Assay of Prazocin and Polythiazide

Prazocin and Polythiazide pure drugs (API), Combination Prazocin and Polythiazide tablets (**MINIZIDE 2**) equivalent to 2 mg and 0.5 mg of Prazocin and Polythiazide respectively was used to perform assay by utilizing the method developed and under the optimized chromatographic conditions. Sample solutions were injected in to the HPLC system and scanned at 270 nm from which the % of drug was estimated.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

Chromatographic conditions

Flow rate : 1 ml/min
Column : Discovary 150 x 4.6 mm, 5µ.
Detector wave length : 270nm
Column temperature : 30°C
Injection volume : 10µL
Run time : 6 min
Diluent : Water: ACN (50:50)

To develop and establish a suitable RP-HPLC method for simultaneous estimation of Prazocin and Polythiazide in bulk and Tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table 1.

The final analysis was performed by using 60% Ortho phosphoric acid:40% Acetonitrile at a flow rate of 1ml/min. samples were analyzed at 270 nm detector wave length and at an injection volume of 10uLusing Discovery 150 x 4.6 mm, 5µ. Column with run time of 6 min. The proposed method was optimized to give sharp peak with good resolution and minimum tailing effect for Prazocin and Polythiazide, the optimized chromatogram was obtained as shown in (Figure-3).

Validation

Linearity was established for Prazocin (10-60µg/ml) and Polythiazide (2.5-15µg/ml) at six different concentrations each were injected in a duplicates and average areas were determined and linearity equations were obtained as Prazocin was y = 50050.x + 7773 and of Polythiazide was y = 95434.x + 6175 , Correlation coefficient (R^2) was determined as 0.999 for the two drugs. The Linearity calibration curves were plotted as shown in (Figure-4&5) for Prazocin and Polythiazide respectively. Retention times of Prazocin and Polythiazide were 2.316 min and 3.176 min respectively. Where no interfering peaks in blank and placebo at retention times of these drugs were not found in this method. So this method holds its specificity.Three levels of Accuracy samples 50%, 100%, 150% were prepared and Triplicates of injections were given for each level of accuracy and mean %Recovery was obtained as 100.49% and 100.40% for Prazocin and Polythiazide respectively were shown in (Table-2).% RSD was calculated from the corresponding peaks obtained by injecting six times a known concentration of Prazocin and Polythiazide the repeatability was obtained as 0.5% and 0.7% respectively for Prazocin and Polythiazide and the % RSD for intermediate Precision was obtained as 1.2%, 1.0% for Prazocin and Polythiazide , Low % RSD values indicates that the method developed was precise as shown in (Table 3).

Table 1. Optimized Chromatographic Conditions

Parameter	Condition
RP-HPLC	WATERS HPLC 2695 SYSTEM equipped with quaternary pumps with PDA DETECTOR
Mobile phase	Buffer and ACN: taken in the ratio 60:40
Flow rate	1ml/min
Column	Discovary 150 x 4.6 mm, 5µ.
Detector wave length	270nm
Column temperature	30°C
Injection volume	10µL
Run time	6 min
Diluent	Water and Acetonitrile in the ratio 50:50
Retention Time	Prazocin 2.316min and Polythiazide 3.176 min
Theoretical Plates	Prazocin 2635 and Polythiazide 2645

Table 2. Accuracy results of Prazocin and Polythiazide

Conc.	Prazocin			Polythiazide		
	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery
	20	20.14206	100.71	5	4.967014	99.34
50%	20	20.0584	100.29	5	5.005962	100.12
	20	20.3218	101.61	5	4.981631	99.63
	40	40.64863	101.62	10	10.04037	100.40
100%	40	40.0815	100.20	10	9.928464	99.28
	40	39.97421	99.94	10	10.16522	101.65
	60	59.63608	99.39	15	15.04307	100.29
150%	60	60.48839	100.81	15	15.14164	100.94
	60	59.88657	99.81	15	15.28911	101.93
	Mean % Recovery		100.49%	Mean % Recovery		100.40%

Table 3. Precision Results of Prazocin and Polythiazide

S.No	Repeatability		Intermediate precision	
	Area of Prazocin	Area of Polythiazide	Area of Prazocin	Area of Polythiazide
1.	2112634	957098	2095436	942671
2.	2103746	960818	2034156	941779
3.	2132907	966473	2054551	955600
4.	2113514	974026	2041885	963678
5.	2105721	968735	2082974	962778
6.	2122924	955776	2041652	957855
Mean	2115241	963821	2058442	954060
S.D	10987.8	7131.1	25025.4	9652.1
%RSD	0.5	0.7	1.2	1.0

Table 4.LOD and LOQ values of Prazocin and Polythiazide

Molecule	LOD	LOQ
Prazocin	0.079	0.240
Polythiazide	0.03	0.08

Table 5. Robustness Data of Prazocin and Polythiazide

S.No.	Condition	%RSD of Prazocin	%RSD of Polythiazide
1	Flow rate (-) 0.9ml/min	1.2	1.2
2	Flow rate (+) 1.0.3ml/min	1.1	1.1
3	Mobile phase (-) 35B:65A	0.7	1.7
4	Mobile phase (+) 45B:55A	0.7	0.7
5	Temperature (-) 25°C	0.9	1.3
6	Temperature (+) 35°C	0.7	1.0

Table 6. System Suitability Parameters Results of Prazocin and Polythiazide

S no	Prazocin			Polythiazide			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.289	2607	1.41	3.142	2538	1.35	3.2
2	2.290	2398	1.43	3.145	2518	1.47	3.2
3	2.293	2590	1.38	3.145	2645	1.35	3.1
4	2.293	2612	1.56	3.147	2607	1.29	3.2
5	2.299	2425	1.46	3.147	2627	1.38	3.1
6	2.316	2635	1.37	3.176	2530	1.30	3.3

Table 7. Assay Results of Prazocin and Polythiazide

S.No	Prazocin			Polythiazide		
	Standard Area	Sample area	% of Drug	Standard Area	Sample area	% of Drug
1.	2113628	2112634	99.49	962165	957098	99.23
2.	2120349	2103746	99.07	967092	960818	99.62
3.	2101349	2132907	100.44	966193	966473	100.21
4.	2119999	2113514	99.53	957087	974026	100.99
5.	2124437	2105721	99.16	962518	968735	100.44
6.	2148889	2122924	99.97	966088	955776	99.10
Mean	2121442	2115241	99.61	963524	963821	99.93
S.D	15686.8	10987.8	0.52	3760.9	7131.1	0.74
%RSD	0.7	0.5	0.52	0.4	0.7	0.74

The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curve Prazocin and Polythiazide. The detection limit values were obtained as 0.079 and 0.03 and Quantitation limit were fund to be 0.240 and 0.08 for Prazocin and Polythiazide Respectively as given in (Table-4). Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit as shown in (Table-5). The system suitability parameters like Retention time, Resolution, USP plate count and peak asymmetry or Tailing evaluated to check whether the results complies the prescribed limits and shown in (Table-6). Prazocin and Polythiazide pure drugs (API), Combination Prazocin and Polythiazide tablets (MINIZIDE 2) equivalent to 2 mg and 0.5 mg of Prazocin and Polythiazide respectively was used to perform assay and the Average % of

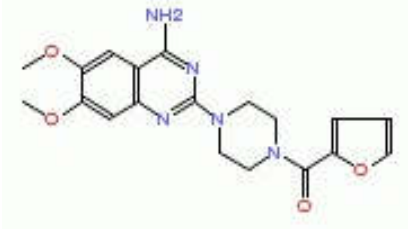

Figure 1. Chemical Structure of Prazocin

Table 8. Degradation Data of Prazocin

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	3.49	0.540	0.741
2	Alkali	2.81	0.526	0.680
3	Oxidation	3.61	0.529	0.735
4	Thermal	4.57	0.422	0.590
5	UV	1.79	0.489	0.619
6	Water	0.84	0.600	1.038

Table 9. Degradation Data of Polythiazide

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	3.64	0.592	0.832
2	Alkali	1.88	0.566	0.752
3	Oxidation	2.90	0.529	0.735
4	Thermal	4.11	0.420	0.637
5	UV	2.44	0.493	0.704
6	Water	0.90	0.515	1.025

drug was found to be 99.61 and 99.93% for Prazocin and Polythiazide respectively the results were shown in (Table-7) and the chromatograms for Prazocin and Polythiazide standard drugs and ophthalmic solution dosage forms were shown in (Figure-6, 7) Respectively.

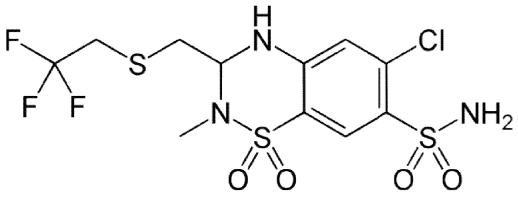

Figure 2. Chemical Structure of Polythiazide

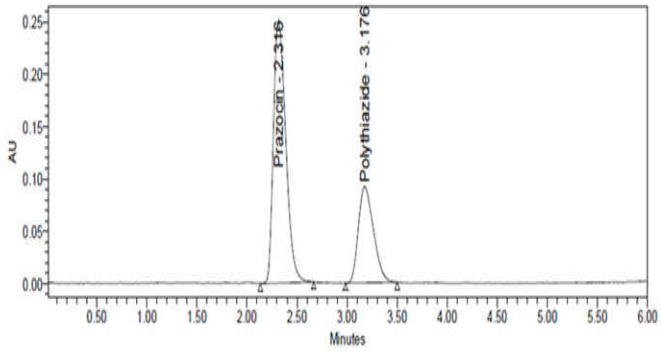

Figure 3. Optimized Chromatogram of Prazocin and Polythiazide

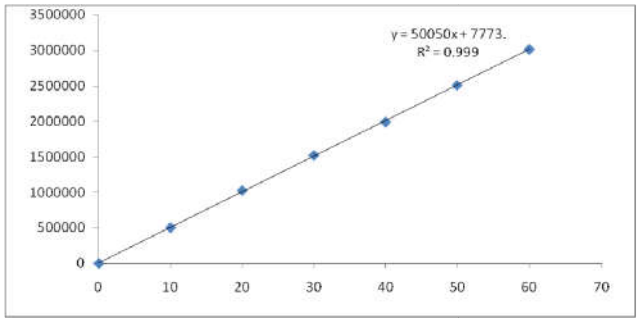

Figure 4. Linearity Curve of Prazocin

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

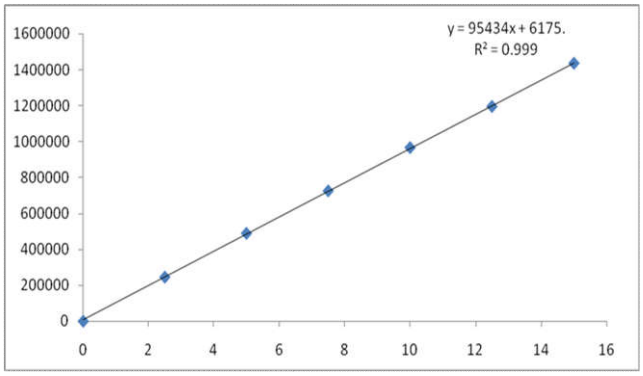

Figure 5. Calibration Curve of Polythiazide

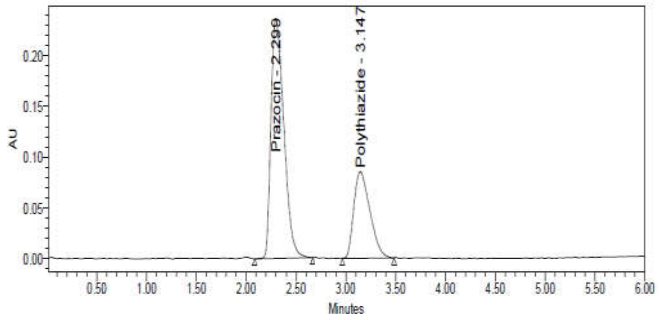

Figure 6. Standard Chromatogram of Prazocin and Polythiazide

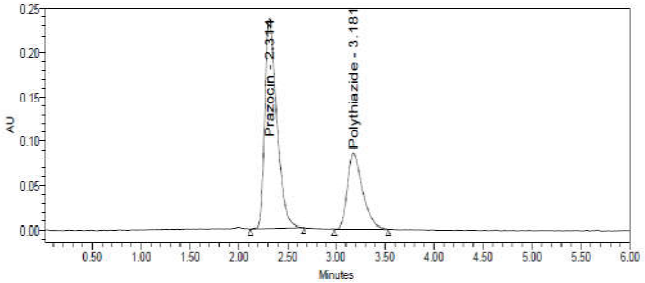

Figure 7. A Sample Chromatogram of Prazocin and Polythiazide Tablet Dosage Form

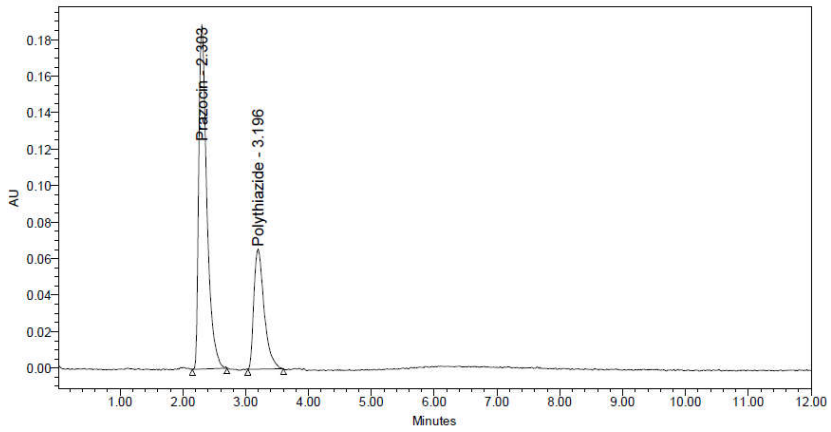

Fig. 8. Acid chromatogram of Prazocin and Polythiazide

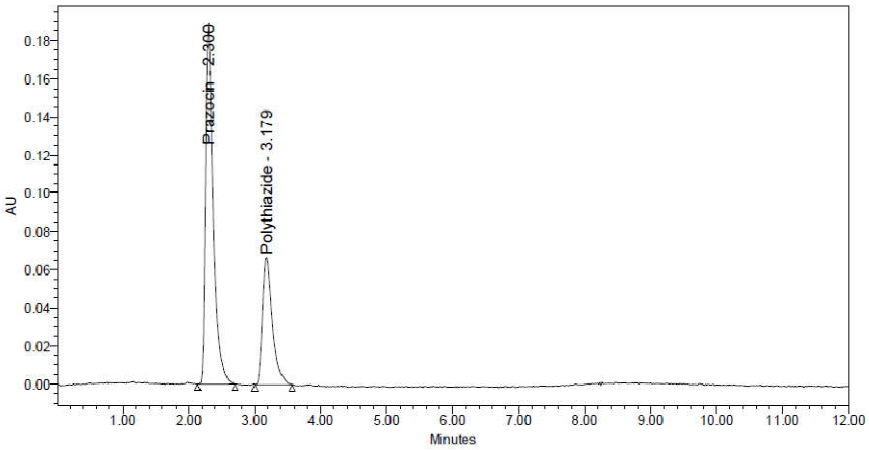

Fig. 9. Base chromatogram of Prazocin and Polythiazide

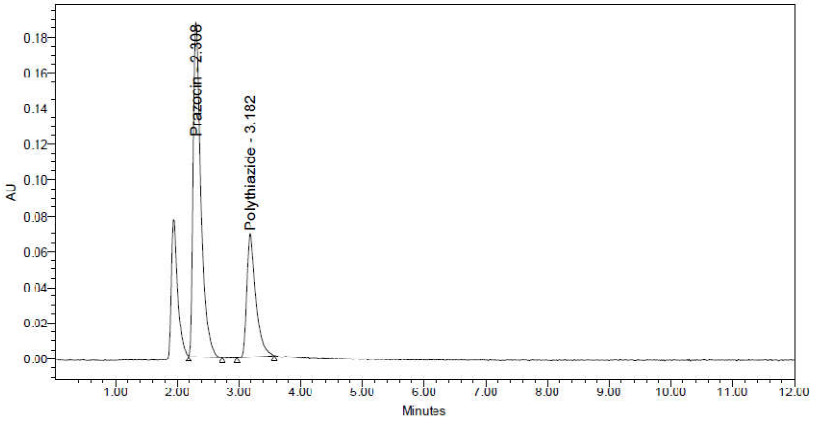

Fig. 10. Peroxide chromatogram of Prazocin and Polythiazide

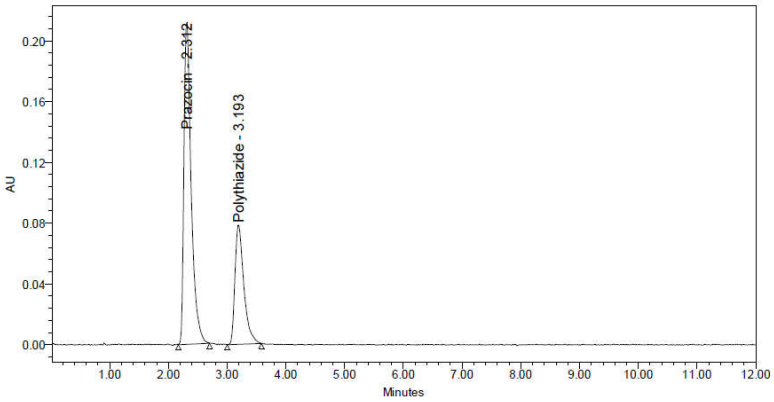

Fig. 11. Thermal chromatogram of Prazocin and Polythiazide

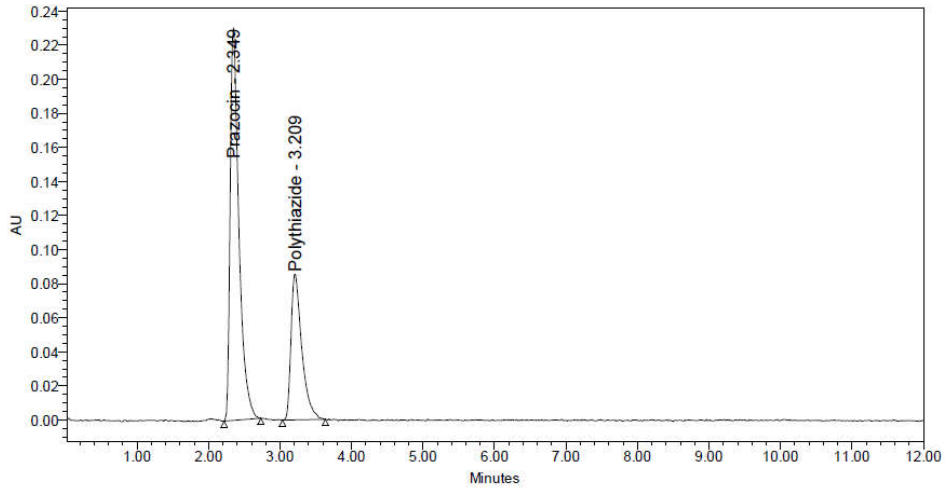

Fig. 12. UV chromatogram of Prazocin and Polythiazide

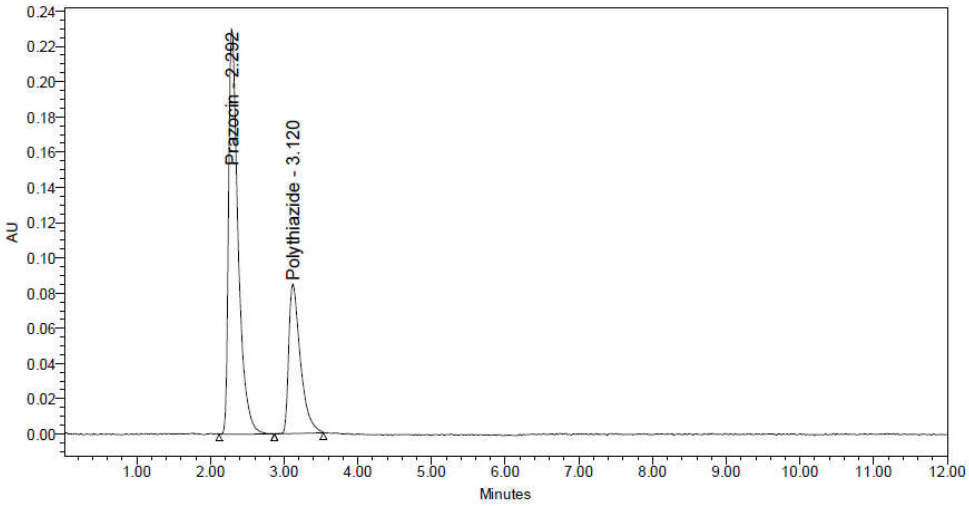

Fig. 13. Water chromatogram of Prazocin and Polythiazide

Conclusion

A new Accurate, Precise, Simple and reliable method for the simultaneous estimation of the Prazocin and Polythiazide in Tablet Dosage Form has been developed. The method developed was validated and was found to be sensitive, accurate, precise and reliable for the analysis of Prazocin and Polythiazide in Bulk and Tablet dosage forms. The Results obtained were within the prescribed limits of ICH Guidelines and shown accuracy and preciseness of the method developed.As the Retention times were decreased and that run time was less the method can be effectively adopted in regular quality control testing in industries which is also economical too. Finally it can be concluded from the results that the method developed was simple and accurate with robust and reliability as added values to the method.

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