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SIMULTANEOUS ESTIMATION OF PRAZOSIN AND POLYTHIAZIDE IN BULK AND FIXED DOSE COMBINATION (TABLETS) BY RP-HPLC

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Abstract:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Prazosin 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 Prazosin and Polythiazide were found to be 2.316 min and 3.176. %RSD of the Prazosin and Polythiazide were found to be 0.5 and 0.7 respectively. %Recovery was obtained as 100.49% and 100.40% for Prazosin and Polythiazide respectively. LOD, LOQ values obtained from regression equations of Prazosin and Polythiazide were 0.079, 0.240 and 0.03, 0.08 respectively. Regression equation of Prazosin 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 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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NTRODUCTION:

Prazosin is indicated for the treatment of hypertension (high blood pressure). Prazosin can be given alone or given with other blood pressure-lowering drugs, including diuretics or beta-adrenergic blocking agents Label. Prazosin does not negatively impact lung function, and therefore may be used to manage hypertension in patients who are asthmatic or patients with chronic obstructive lung disease It belongs to the class of drugs known as alpha-1 antagonists.[1] Recently, many studies have evaluated the benefits of this drug in controlling the symptoms of post-traumatic stress disorder (PTSD) and associated nightmares.[2] IUPAC name 2-[4-(furan-2-carbonyl) piperazin-1-yl]-6,7-dimethoxyquinazolin-4-amine. Chemical formula $C_{19}H_{21}N_5O_4$. Molecular weight 383.41. Prazosin (hydrochloride is soluble in the organic solvent DMSO at a concentration of approximately. 0.1 mg/ml.

Polythiazide a thiazide diuretic with actions and uses similar to those of hydrochlorothiazide. As a diuretic, polythiazide inhibits active chloride reabsorption at the early distal tubule via the thiazide-sensitive Na-Cl cotransporter (TSC), resulting in an increase in the excretion of sodium, chloride, and water. Thiazides like polythiazide also inhibit sodium ion transport across the renal tubular epithelium through binding to the thiazide sensitive sodium-chloride transporter. This results in an increase in potassium excretion via the sodium-potassium exchange mechanism. The antihypertensive mechanism of polythiazide may be mediated through its action on carbonic anhydrases in the smooth muscle or through its action on the large-conductance calcium-activated potassium (KCa) channel, also found in the smooth muscle. IUPAC name 6-chloro-2-methyl-1,1-dioxo-3-{[(2,2,2-trifluoroethyl) sulfanyl]methyl}-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide Chemical formula $C_{11}H_{13}ClF_3N_3O_4S_3$ Molecular weight 439.88.

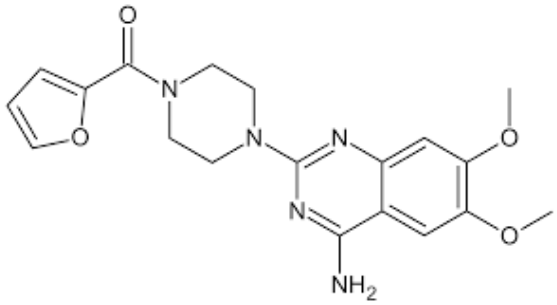


Figure 1: Structure of Prazosin

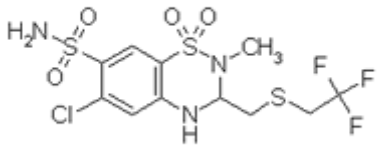


Figure 2: Structure of Polythiazide

Prazosin and Polythiazide is a fixed dose combination drug for treatment of hypertension. Literature review reveals that very few analytical methods has been reported for the determination of Prazosin and Polythiazide individually and with other combinations which includes high performance liquid chromatography (HPLC)[3-19], UV-Visible-Spectrophotometric[20-23] and LC-MS[24,25]. The present study was intended to develop a new validated method for the simultaneous estimation of Prazosin and Polythiazide with forced degradation studies as per ICH guidelines[26].

MATERIALS AND METHODS:

Chemicals and Reagents: Prazosin and Polythiazide were obtained as a gift sample from Sun pharma India Pvt. Ltd, Hyderabad. Sodium hydroxide, hydrochloric acid, Methanol for HPLC (Merck), Acetonitrile for HPLC (Merck) and Water for HPLC (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a HPLC equipped with Auto Sampler and PDA Detector and Empower 2 software. Analysis was carried out at 270 nm with column Discovary 150 x 4.6 mm, 5µ dimensions at 0^0C temperature. The optimized mobile phase consists of0.1% Ortho phosphoric: Acetonitrile in the proportion of 60:40, Flow rate was maintained at 1.0 ml/min and run time for 6 min at Temperature 30 ° C

Preparation of solutions:

Preparation of buffer:

0.1% OPA Barrier: 1ml of ortho phosphoric acid was watered down to 1000ml with HPLC quality water.

Preparation of mobile phase:

Mobile stage was prepared my mixing 0.1% Ortho phosphoric: Acetonitrile in the ratio of 60:40 and also sonicated making use of ultrasonic bathroom to degas as well as based on vacuum purification with 0.45 Millipore Nylon filter.

The diluents:
The Mobile phase was used as the diluent.

Preparation of standard stock solution:
Precisely gauged 10mg of Prazosin, 1mg of Polythiazide as well as moved to 10ml and also 10ml specific volumetric flagons as well as 3/4 th of diluents was included in this pitcher as well as sonicated for 10 minutes. Container were made up with diluents and also named as Basic supply arrangement. (400µg/ ml of Prazosin as well as 100µg/ ml Polythiazide)..

Preparation of Sample stock solution:
5 tablet computers were assessed and also the regular tons of every tablet was established, at that point the weight comparable to 1 tablet was relocated right into a 10ml volumetric carafe, 5ml of diluents was included and also sonicated for 25 min, even more the quantity was made up with diluent and looked by HPLC networks (400µg/ ml of Prazosin as well as 10µg/ ml of Polythiazide).

Procedure: 10 µL of standard and sample solutions were injected into the LC-system and measure the peak areas for Prazosin and Polythiazide.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.
System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 6 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Inertsil Discovery (150 x 4.6 mm, 5), the mobile phase of composition Water: ACN (50:50) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Prazosin and Polythiazide in their tablet dosage form. The result obtained for Prazosin and Polythiazide was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity and Range: Stock solution was prepared by dissolving the appropriate amount of Prazosin and Polythiazide in 10 ml of diluent and further diluted to the required concentrations with diluent. The solution was prepared at five concentration levels ranging from 10 µg/ml to 50 µg/ml of Prazosin and 20 µg/ml to 100 µg/ml of Polythiazide. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for v Prazosin and Polythiazide and calculate the individual recovery and mean recovery values. The resulte are shown in table 4.

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 5.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 6.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1ml/min. The resulte are shown in table 7.

LOD and LOQ: The sensitivity of RP-UPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The resulte are shown in table 8.

$LOD = 3.3\sigma/S$ and
$LOQ = 10\ \sigma/S$, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

RESULTS AND DISCUSSION:

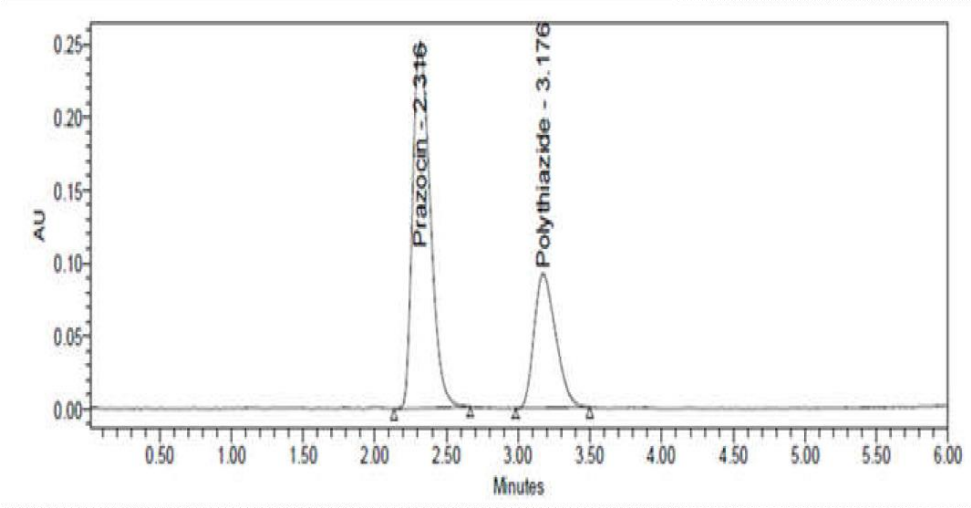


Figure 3: Standard chromatogram

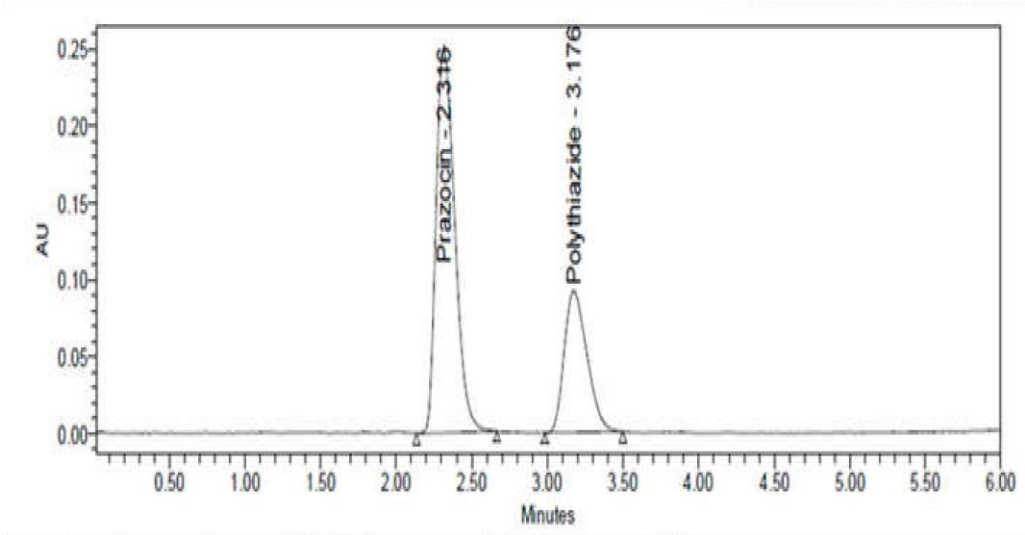


Figure 4: Sample chromatogram

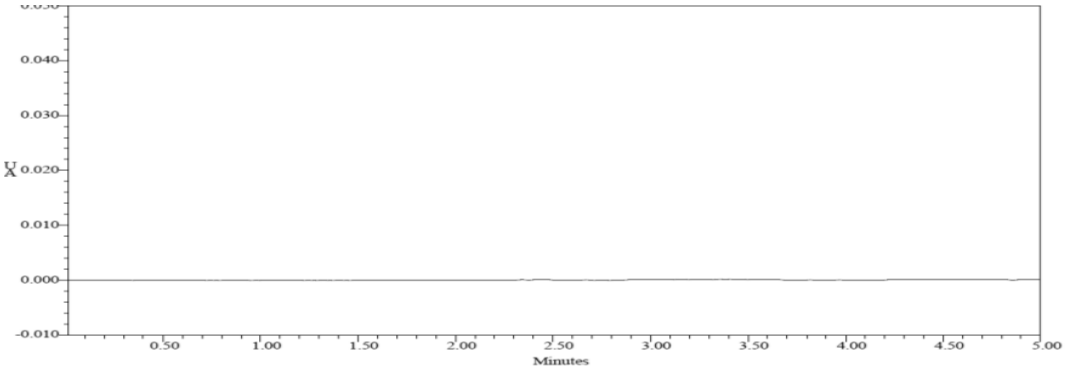


Figure 5: Blank chromatogram

Table 1: System suitability parameter

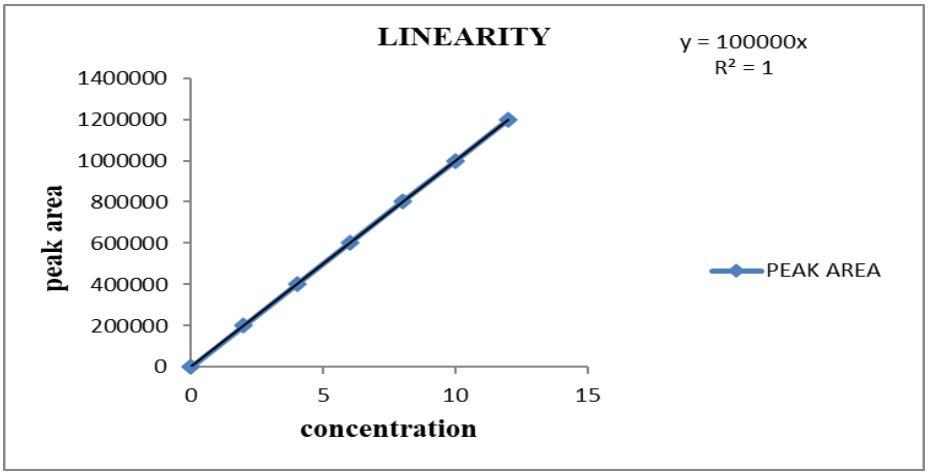
S no	Prazosin			Polythiazide			
Inj	RT(min)	USP Plate Count	Tailing	RT(min	USP Plate Count	Tailing	Resolution
1	2.289	2607	1.41	3.142	2538	1.35	3.2
2	2.290	2398	1.43	3.145	2518	1.47	3.2
3	2.293	2590	1.38	3.145	2645	1.35	3.1
4	2.293	2612	1.56	3.147	2607	1.29	3.2
5	2.299	2425	1.46	3.147	2627	1.38	3.1
6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

Table 2: Assay results for Prazosin and Polythiazide

	Label Claim (mg)	% Assay
Prazosin	10	99.61
Polythiazide	1	99

Table 3: Linearity results for Prazosin and Polythiazide

Prazosin		Polythiazide	
Concentration(µg/ml)	Area	Concentration(µg/ml)	Area
10	185689	20	665985
20	349852	40	1298698
30	521541	60	1927852
40	685986	80	2548545
50	848265	100	3162468
Correlation coefficient	0.999	Correlation coefficient	0.999



Calibration Curve of Prazosin

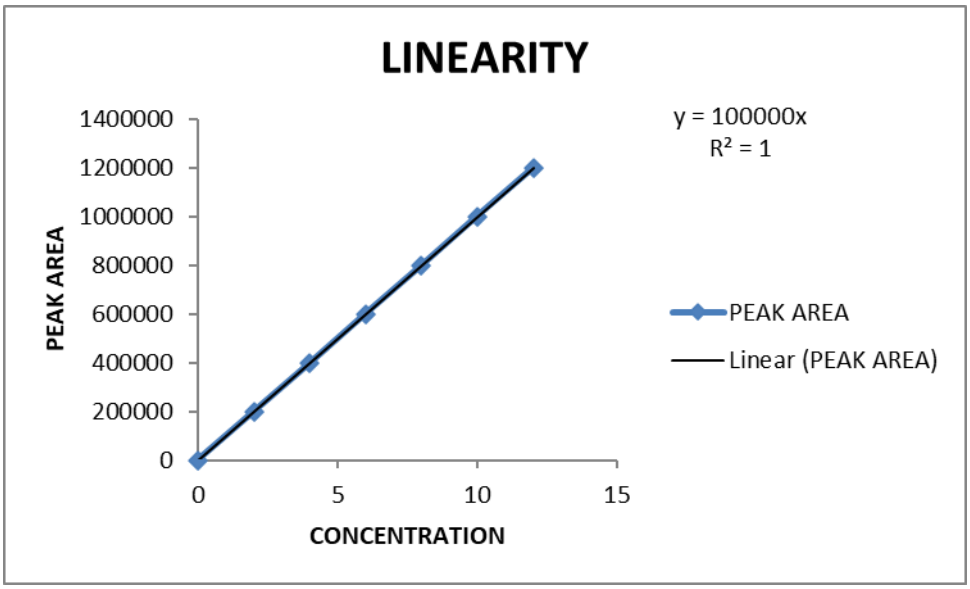


Figure 8: Robustness results of Polythiazide

Table 4: Accuracy results of Prazosin and Polythiazide

Conc	Prazosin			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

Table 5: Precision results for Prazosin and Polythiazide

S.No	Repeatability		Intermediate precision	
	Area of Prazosin	Area of Polythiazide	Area of Prazosin	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 6: Intermediate precision resultes for Prazosin and Polythiazide:

Injection	Area for Prazosin	Area for Polythiazide
Injection-1	536598	1658254
Injection-2	536985	1659872
Injection-3	534587	1653589
Injection-4	536985	1658458
Injection-5	536985	1653652
Injection-6	538568	1652395
Average	536784.7	1656037
STD Deviation	1277.909	3175.804
%RSD	0.238067	0.191771

Table 7. Robustness Data of Prazosin and Polythiazide

s.no	Condition	%RSD of Prazosin	%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 8: LOD, LOQ of Prazosin and Polythiazide

Drug	LOD	LOQ
Prazosin	0.079 µg/mL	0.24 µg/mL
Polythiazide	0.03 µg/mL	0.08 µg/Ml

CONCLUSION:

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Prazosin and Polythiazide in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Prazosin and Polythiazide in pure and its pharmaceutical dosage forms.

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