ORIGINAL ARTICLE

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

G. Dharmamoorthy[1], K. S. Nataraj[2], A. Krishna Manjari Pawar[3]

[1]Research Scholar, Andhra University, Visakhapatnam, Andhrapradesh, India, [2]Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Bhimavaram, Andhra Pradesh, India, [3]Department of Pharmaceutical Analysis, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India

Abstract

Objective: A new simple and precise stability-indicating bioanalytical reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of prazosin and polythiazide in their formulation and in human plasma. **Materials and Methods**: The developed method was successfully used for assaying drug contents in plasma. Isocratic elution mode was carried at Agilent C18 column (150 mm × 4.6 mm, 5 µm particle size) using 0.01 N disodium hydrogen phosphate pH 5.0:acetonitrile (55:45) as mobile phase at flow rate 1.0 ml min at detection wavelength 265 nm. Celecoxib was taken as an internal standard. The method was validated as per ICH guidelines. **Results and Discussion:** The present validated method can be successfully applied for the estimation of prazosin and polythiazide in human plasma over the concentration range of 12.5–500 ng/m of prazosin and 6.25–250 ng/ml of polythiazide. The method for the determination of prazosin and polythiazide in human plasma using HPLC detection met the acceptance criteria with respect to selectivity, precision, accuracy, linearity, and recovery. **Conclusion:** The proposed method is simple, rapid, accurate, precise, and appropriate for pharmacokinetic and therapeutic drug monitoring in the clinical laboratories.

Key words: Celecoxib internal standard, ICH guidelines, prazosin and polythiazide K_2EDTA plasma, reverse-phase high-performance liquid chromatography, validation

INTRODUCTION

Prazosin is an antihypertensive agent which lowers arterial blood pressure by effecting blockade of vascular post-junctional α-adrenoreceptors[1-3] and is the first of such agents to show long-term efficacy in the treatment of hypertension. Alpha-adrenergic receptors are essential for the regulation of blood pressure in humans. Two types of alpha receptors, alpha 1 and alpha 2, both play a role in regulating blood pressure. Alpha-1 receptors are postsynaptic (located after the nerve junction or space between a nerve fiber and target tissue).

Polythiazide is a diuretic with actions and uses similar to those of hydrochlorothiazide.[4] As a thiazide diuretic, polythiazide inhibits the sodium-chloride symporter which decreases solute reabsorption leading to a retention of water in the urine, as water normally follows solutes. The short-term antihypertensive action is based on the fact that thiazides decrease preload, decreasing blood pressure, polythiazide also inhibits 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 through the sodium-potassium exchange mechanism.

Address for correspondence:
G. Dharmamoorthy, Research Scholar, Andhra University, Visakhapatnam - 530 003,
Andhra Pradesh, India. Phone: +91-9603774847.
E-mail: dharmamoorthy111@gmail.com

Received: 28-03-2020
Revised: 14-04-2020
Accepted: 19-04-2020

Table 1: List of chemicals and solvents

Chemical name	Grade	Manufacturing company
Distilled water HPLC water	HPLC	Rankem, Avantor Performance Materials India Limited
HPLC water	Analytical reagent	Rankem, Avantor Performance Materials India Limited
Acetonitrile	Analytical reagent	Rankem, Avantor Performance Materials India Limited
Phosphate buffer	Analytical reagent	Rankem, Avantor Performance Materials India Limited
Methanol	Analytical reagent	Rankem, Avantor Performance Materials India Limited
Sodium dihydrogen phosphate	Analytical reagent	Rankem, Avantor Performance Materials India Limited
Orthophosphoric acid	Analytical reagent	Rankem, Avantor Performance Materials India Limited

HPLC: High-performance liquid chromatography

Table 2: List of instruments

Instrument	Company name	Brand name
Electronic balance	Sartorius	Denver
pH meter	Metsar	BVK enterprises
Sonicator	Lab man	BVK enterprises
Centrifuge	Thermo fisher	-
Vertex	Remi CM101	-
HPLC water	Alliance	Water HPLC 2695 SYSTEM

HPLC: High-performance liquid chromatography

Extensive survey of literature few methods has been reported for the simultaneous estimation of prazosin and polythiazide using spectroscopic and chromatographic methods.[5-8] However, there is no reported for the bioanalytical methods. The main aim of the present study is to develop a precise, sensitive, accurate, selective, reproducible, and rapid bioanalytical technique for the estimation of prazosin and polythiazide in human plasma[9-13] and validated as per ICH guidelines.[14,15]

MATERIALS AND METHODS

Materials

List of chemicals and solvents [Table 1] and solvents [Table 2] given below.

API

Prazosin and polythiazide, API, were gifted by BMR Chemicals, Hyderabad.

Human plasma

K_2 EDTA control plasma procured by Deccan Pathological Labs, Hyderabad.

Method development

Diluent

Based on the solubility of the drugs, diluent was selected, 0.01 N disodium hydrogen orthophosphate and acetonitrile taken in the ratio of 50:50.

Preparation of prazosin stock solution (25 µg/ml)

Take 2.5 mg of prazosin in 100 ml volumetric flask and make the volume with diluent to produce 25 µg/ml.

Table 3: System suitability of prazosin

Analyte	Prazosin	ISTD		Celecoxib		
Sample name		Analyte area	Analyte RT (min)	ISTD Area	ISTD RT (min)	Area ratio
AQ MQC		29,558	3.98	69,634	2.683	0.4245
AQ MQC		29,056	4.01	69,452	2.684	0.4184
AQ MQC		29,993	4.02	69,667	2.688	0.4305
AQ MQC		29,390	4.02	69,151	2.697	0.4250
AQ MQC		29,884	4.06	69,724	2.723	0.4286
AQ MQC		29,717	4.08	69,956	2.724	0.4248
Mean±SD			4.029±0.0357		2.700±0.0190	0.42529±0.004179
%CV			0.89		0.70	0.98

RT: Retention time, MQC: Middle quality control

Table 4: System suitability of polythiazide

System suitability						
Validation No.		**SOP No.**			**Column ID.**	
Analyte	**Polythiazide**	**ISTD**		**Celecoxib**		
Acquisition batch ID					**Date**	
Sample name		**Analyte area**	**Analyte RT (min)**	**ISTD area**	**ISTD RT (min)**	**Area ratio**
AQ MQC		16,193	4.89	69,634	2.683	0.2325
AQ MQC		16,231	4.93	69,452	2.684	0.2337
AQ MQC		16,028	4.95	69,667	2.688	0.2301
AQ MQC		16,141	4.96	69,151	2.697	0.2334
AQ MQC		16,041	5.01	69,724	2.723	0.2301
AQ MQC		16,118	5.05	69,956	2.724	0.2304
Mean±SD			4.966±0.0570		2.700±0.0190	0.23170±0.001714
%CV			1.15		0.70	0.74

RT: Retention time, MQC: Middle quality control

Table 5: Auto-sampler carryover of prazosin

Acquisition batch ID			**Date**	
Sample ID	**Peak area**		**% carryover**	
	Drug	**ISTD**	**Drug**	**ISTD**
Unextracted samples				
RS	0	0	N/A	N/A
AQ ULOQ	61,732	70,558	0.00	0.00
RS	0	0		
AQ LLOQ	1515	71,349	N/A	N/A
Extracted samples				
STD Blk	0	0	N/A	N/A
ULOQ	59,694	69,246	0.00	0.00
STD Blk	0	0		
LLOQ	1489	69,231	N/A	N/A

LLOQ: Lower limit of quantitation

Table 6: Auto-sampler carryover of polythiazide

Acquisition batch ID			**Date**	
Sample ID	**Peak area**		**% carryover**	
Drug	**ISTD**	**Drug**	**ISTD**	
Unextracted samples				
RS	0	0	N/A	N/A
AQ ULOQ	33,275	70,558	0.00	0.00
RS	0	0		
AQ LLOQ	844	71,349	N/A	N/A
Extracted samples				
STD Blk	0	0	N/A	N/A
ULOQ	32,502	69,246	0.00	0.00
STD Blk	0	0		
LLOQ	818	69,231	N/A	N/A

LLOQ: Lower limit of quantitation

Preparation of prazosin spiking solutions

From the above prazosin stock solution, 0.05 ml, 0.1 ml, 0.15 ml, 0.6 ml, 1.0 ml, 1.2 ml, 1.6 ml, and 2.0 ml were pipette and transferred to eight individual 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 0.125 µg/ml, 0.25 µg/ml, 0.375 µg/ml, 1.0 µg/ml, 2.5 µg/ml, 3.0 µg/ml, 4.0 µg/ml, and 5.0 µg/ml. Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 0.0125 µg/ml, 0.025 µg/ml, 0.0375 µg/ml, 0.10 µg/ml, 0.25 µg/ml, 0.3 µg/ml, 0.4 µg/ml, and 0.5 µg/ml.

Preparation of polythiazide stock solution (12.5 µg/ml)

Take 1.25 mg of polythiazide in 100 ml volumetric flask and make the volume with diluent to produce 12.5 µg/ml.

Preparation of polythiazide spiking solutions

From the above polythiazide stock solution, 0.05 ml, 0.1 ml, 0.15 ml, 0.6 ml, 1.0 ml, 1.2 ml, 1.6 ml, and 2.0 ml were pipette and transferred to eight individual 10 ml volumetric flask and make up the volume up to the mark with diluent to produce 0.0625 µg/ml, 0.125 µg/ml, 0.1875 µg/ml, 0.5 µg/ml, 1.25 µg/ml, 1.5 µg/ml, 2.0 µg/ml, and 2.5 µg/ml. Calibration standards and QC samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 0.00625 µg/ml, 0.0125 µg/ml, 0.01875 µg/ml, 0.05 µg/ml, 0.125 µg/ml, 0.15 µg/ml, 0.20 µg/ml, and 0.25 µg/ml.

Preparation of internal standard solution (celecoxib)

- Stock-1: Take 5 mg of celecoxib in 100 ml volumetric flask and make up the volume with diluent to produce 50 µg/ml

Table 7: Matrix factor evaluation of prazosin (absence of matrix factor)

Acquisition batch ID		Date	
S. No.	Plasma Lot No.	HQC	LQC
		Nominal concentration (ng/mL)	
		400.000	37.500
		Nominal concentration range (ng/mL)	
		(340.000–460.000)	(31.875–43.125)
		Calculated concentration (ng/mL)	
1	LOT1	404.00	37.55
		401.00	37.86
		398.00	37.87
2	LOT2	402.00	37.86
		396.00	37.90
		398.00	37.14
3	LOT3	403.00	36.91
		405.00	37.88
		400.00	37.78
4	LOT4	399.00	37.91
		398.00	36.84
		405.00	37.98
5	LOT5	399.00	37.86
		398.00	37.79
		402.00	37.80
6	LOT6	404.00	37.94
		401.00	37.00
		406.00	37.99
n		18	18
Mean±SD		401.0556±2.97978	37.6589±0.39279
% CV		0.74	1.04
% mean accuracy		100.26	100.42
Number of QC failed		0	0

LQC: Low-quality control, HQC: High-quality control

- Stock-2: From the above solution, take 1 ml of solution into 10 ml volumetric flask and make up the volume with diluent to produce 5 µg/ml solutions.

Final concentration

From the above solution, take 0.5 ml of solution and spiking blank plasma with working stock dilutions of analyte to produce 1 µg/ml Internal standard solution (ISD) concentration.

Extraction procedure

Take 750 µl of plasma and 500 µl of internal standard, 250 µl of prazosin from the spiking solutions of both into a centrifuging tube and add 1 ml of acetonitrile go for cyclomixer for 15 s. Then vertex for 2 min and finally centrifuge for 5 min at 3200 rpm speed. After the centrifugation, collect the sample and filter it directly inject 50 µL into high-performance liquid chromatography (HPLC).

Optimized chromatographic conditions

Mobile phase	: 0.01 N disodium hydrogen orthophosphate pH (5.0): acetonitrile (55:45)
Flow rate	: 1.0 ml/min
Column	: Agilent C18 (150 mm × 4.6 mm, 5 µ)
Detector wavelength	: 265 nm
Column temperature	: 30°C
Injection volume	: 50 µL

Table 8: Matrix factor evaluation of polythiazide (absence of matrix factor)

Acquisition batch ID		Date	
S. No.	Plasma Lot No.	HQC	LQC
		Nominal concentration (ng/mL)	
		200.000	18.750
		(170.000–230.000)	(15.938–21.563)
		Calculated concentration (ng/mL)	
1	LOT1	196.463	18.180
		199.621	18.201
		192.325	18.155
2	LOT2	201.925	18.766
		201.899	17.891
		200.946	18.155
3	LOT3	199.942	17.974
		200.908	18.762
		199.889	18.690
4	LOT4	201.906	19.083
		200.896	18.858
		202.119	18.016
5	LOT5	200.601	19.002
		196.902	19.095
		198.914	19.092
6	LOT6	201.884	18.760
		200.954	18.682
		200.921	18.488
n		18	18
Mean±SD		199.9453±2.49617	18.5472±0.41815
% CV		1.25	2.25
% mean accuracy		99.97	98.92
Number of QC failed		0	0

LQC: Low-quality control, HQC: High-quality control

Method validation

System suitability

All the system suitability parameters were within the range and satisfactory as per ICH guidelines. The % CV for system suitability test was in the range of 0.89 for retention time (RT) of prazosin, 1.15 for RT of polythiazide, and 0.74% for the area ratio (analyte area/IS area) of celecoxib.

Auto-sampler carryover test

The carry over effect due to the auto sampler was investigated by injecting the sequence of un extracted and extracted samples. Results demonstrated that no significant carry over was observed during this experiment

Matrix factor evaluation

Matrix effect is played a key role in the assessment of pharmacokinetic studies. It was expressed as internal standard normalized matrix factor and it was varied from 0.90 to 0.99 which was close to 1 which indicates that there is no ionization suppression or enhancement in plasma samples.

Table 9: Linearity of prazosin

Final conc. in µg/m	ISD (area)	Drug (area)	Area ratio
0.0125	69,638	1496	0.021
0.025	69,756	2981	0.043
0.0375	69,793	4478	0.064
0.1	69,586	11,936	0.172
0.25	69,547	29,828	0.429
0.3	69,684	35,765	0.513
0.4	69,726	47,735	0.685
0.5	69,675	58,672	0.842

QC samples

The chromatography observed during the course of prazosin and polythiazide which was acceptable and representative chromatograms of standard blank, standard zero (standard blank with internal standard) QC-lower limit of quantitation (LLOQ), QC-L, QC-M1, QC-M2, and QC-H samples.

Table 10: Linearity of polythiazide

Final conc. in µg/m	ISD (area)	Drug (area)	Area ratio
0.00625	69,638	811	0.0116
0.0125	69,756	1236	0.0177
0.01875	69,793	2495	0.0357
0.05	69,586	6586	0.0946
0.125	69,547	16,438	0.2364
0.15	69,684	19,478	0.2795
0.2	69,726	25,234	0.3619
0.25	69,675	31,679	0.4547

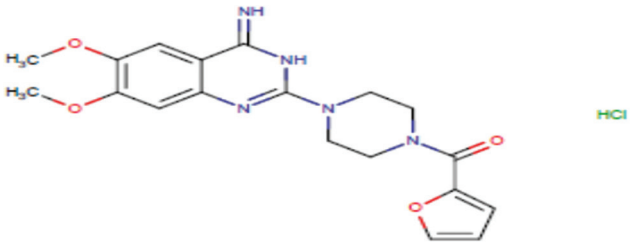

Figure 1: Chemical structure of prazosin

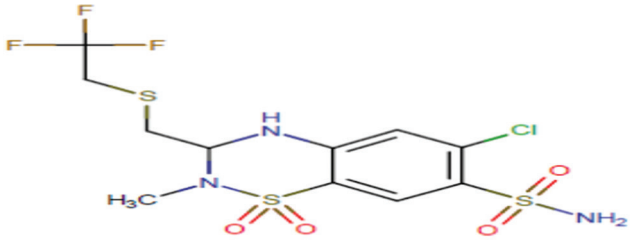

Figure 2: Chemical structure of polythiazide

Selectivity/specificity

To establish the selectivity of the method, possible interference at the RT of prazosin, polythiazide, and internal standard due to endogenous plasma components was checked during the validation. Selectivity was performed by testing six batches of K_2EDTA blank plasma and the mass detection of extracted blank plasma gave good selectivity of both drug and internal standard. No interferences were found at the RTs of analytes and internal standard.

Linearity

Calibration was found to be linear over the concentration range of 0.0125–0.5 µg/ml for prazosin and 0.00625–0.25 µg/ml polythiazide. The coefficient correlation (r^2) value was found consistently greater than 0.999 in all the cases. This indicates linearity of results and an excellent correlation between peak area ratios for each concentration of analytes.

Precision and accuracy

The intraday and interday accuracy and precision were assessed by analyzing six replicates at five different QC levels such as LLOQ, low QC (LQC), middle QC (MQC), and high QC (HQC). Accuracy and precision method performance were evaluated by determined by six replicate analyses for prazosin at four concentration levels, i.e., 0.0125 µg/ml (LLOQ), 0.0375 µg/ml (LQC), 0.25 µg/ml (MQC), and 0.40 µg/ml (HQC), polythiazide at 0.00625 µg/ml (LLOQ), 0.01875 µg/ml (LQC), 0.125 µg/ml (MQC), and 0.2 µg/ml (HQC), the intraday and interday accuracy of plasma samples were assessed and excellent mean % accuracy was obtained with range varied from 98.16 to 100.42% and 98.75 to 100.41% for intraday and 98.83 to 100.03 and 99.70 to 99.86 for interday, respectively. The precision (%CV) of the analytes and plasma samples were calculated and found to be 0.70–4.34% and 0.39–2.73% for intraday and 0.21–2.83% and 0.22–0.74% for interday, respectively.

Recovery

Recovery was determined by measuring the peak areas obtained from prepared plasma samples with those

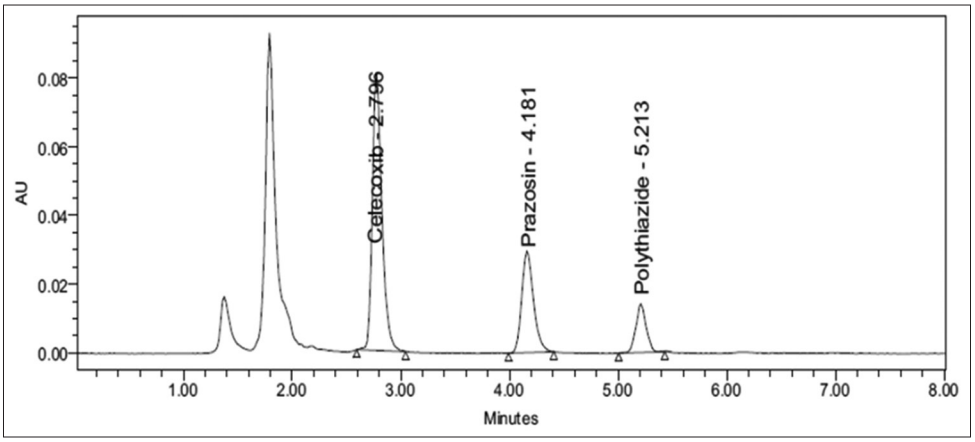

Figure 3: Chromatogram of standard zero sample

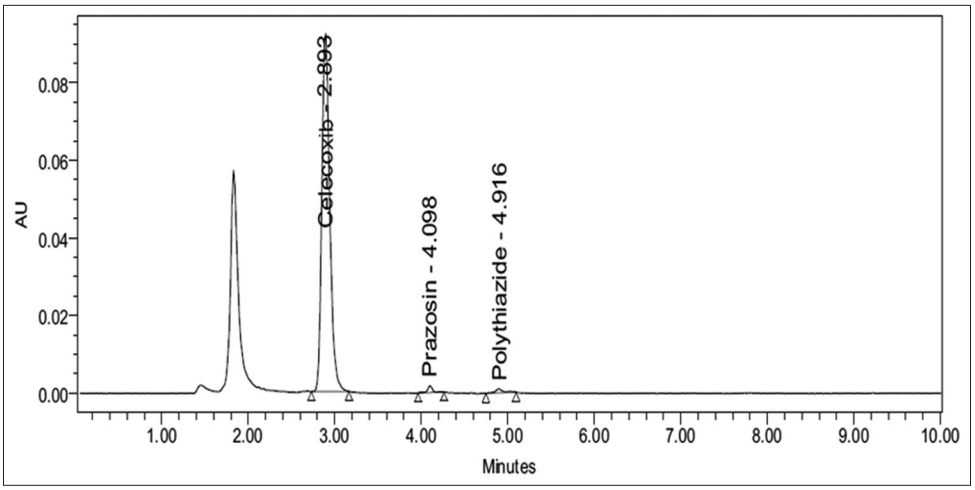

Figure 4: Chromatogram of quality control-lower limit of quantitation sample polythiazide and prazosin

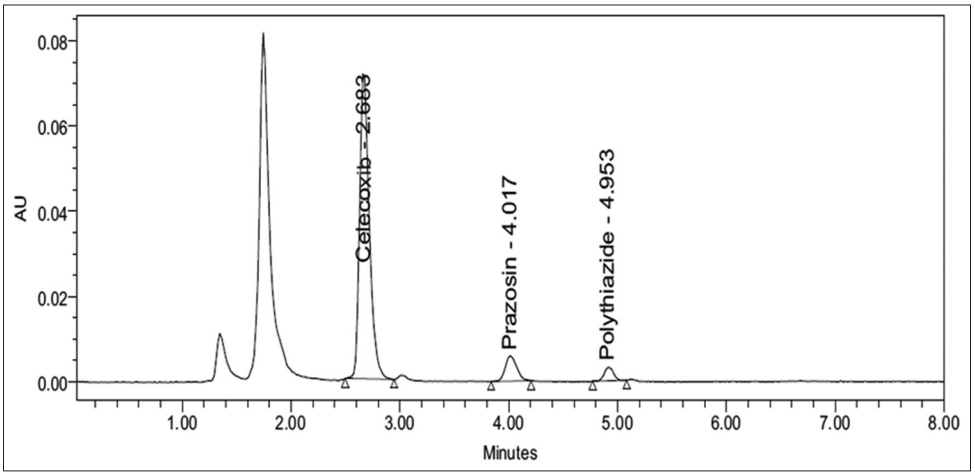

Figure 5: Chromatogram of quality control-low-quality control sample prazosin and polythiazide

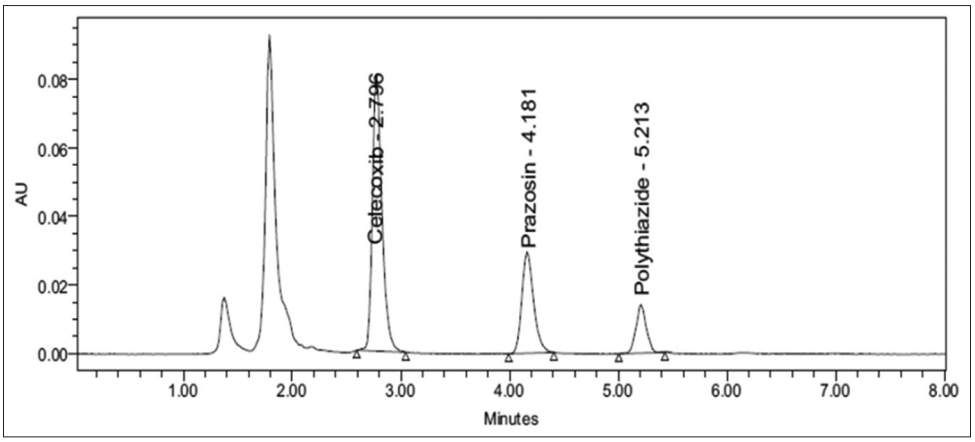

Figure 6: Chromatogram of quality control-middle quality control sample prazosin and polythiazide

extracted blank plasma spiked with standards containing the same area with known amount of prazosin and polythiazide. The overall % mean recovery for prazosin and polythiazide was found to be 98.16% and 98.23%. The overall % mean recovery for celecoxib was found to be 98.11%.

Long-term stock solution stability for polythiazide

In bench-top stability, six replicates of LQC and HQC samples (0.01875 and 0.2 µg/ml) were analyzed for 9 h at room temperature on the laboratory bench. The % mean stability was calculated and found to 99.95% for LQC and 99.87% for HQC, respectively.

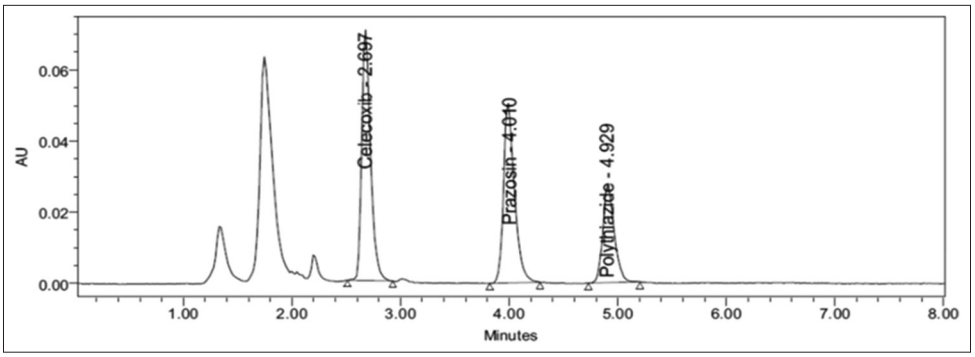

Figure 7: Chromatogram of quality control-high-quality control sample prazosin and polythiazide

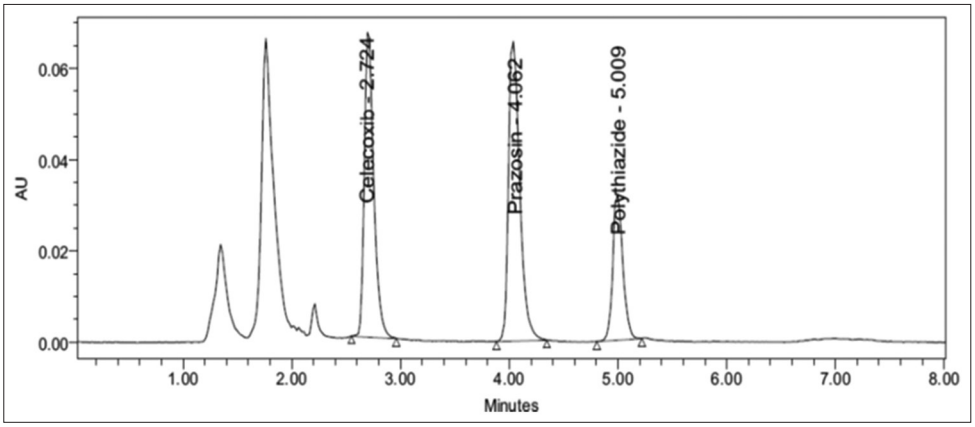

Figure 8: Chromatogram of ULOQ sample prazosin and polythiazide

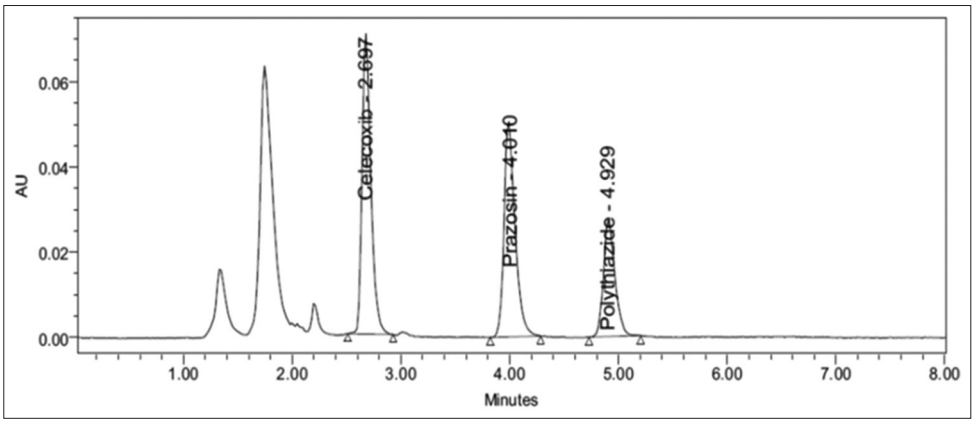

Figure 9: Chromatogram of prazosin and polythiazide

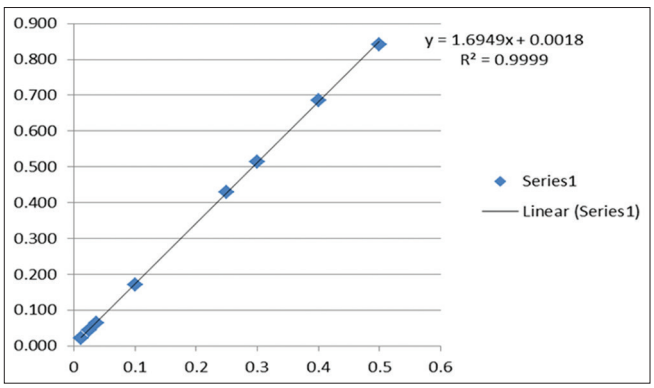

Figure 10: Calibration curve of prazosin

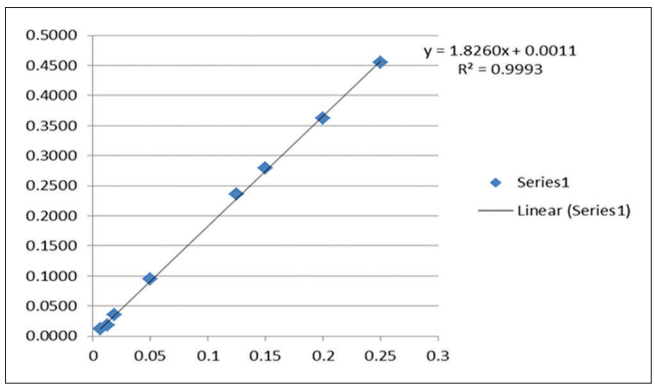

Figure 11: Calibration curve of polythiazide

Table 11: Precision and accuracy results for prazosin

Acquisition batch ID	Date	HQC	MQC1	LQC	LLOQ QC
		Nominal concentration (ng/mL)			
		400.000	250.000	37.500	12.500
		Nominal concentration range (ng/mL)			
		(340.000–460.000)	(212.500–287.500)	(31.875–43.125)	(10.000–15.000)
		Back calculated concentration (ng/mL)			
		399	250.900	37.920	12.289
		400	246.300	37.926	11.268
		400	251.500	38.900	12.330
		400	252.100	36.850	12.324
		400	248.800	37.880	12.288
		402	250.900	37.840	12.313
n		6	6	6	6
Mean±SD		400.0167±0.88412	250.0833±2.16187	37.8860±0.64906	12.1353±0.42526
% CV		0.22	0.86	1.71	3.50
% mean accuracy		100.00	100.03	101.03	97.08
		400	250.800	37.900	12.330
		401	250.100	37.910	12.280
		400	244.010	37.920	11.322
		389	252.300	38.860	12.295
		400	252.700	36.880	12.310
		399	253.600	36.630	11.268
n		6	6	6	6
Mean±SD		397.9167±4.65507	250.5850±3.46459	37.6833±0.81163	11.9675±0.52146
% CV		1.17	1.38	2.15	4.36
% mean accuracy		99.48	100.23	100.49	95.74
		400	256.100	37.880	12.908
		402	250.500	36.940	12.920
		400	250.300	37.860	12.990
		399	250.800	36.880	11.980
		399	250.600	37.910	12.810
		399	250.900	36.930	12.699
n		6	6	6	6
Mean±SD		399.7867±1.02033	251.5333±2.24737	37.4000±0.53009	12.7178±0.37533
% CV		0.26	0.89	1.42	2.95
% mean accuracy		99.95	100.61	99.73	101.74
Between batch precision and accuracy					
n		18	18	18	18
Mean±SD		399.2400±2.80107	250.7339±2.60255	37.6564±0.66511	12.2736±0.53298
% CV		0.70	1.04	1.77	4.34
% mean accuracy		99.81	100.29	100.42	98.19

LLOQ: Lower limit of quantitation, LQC: Low-quality control, MQC: Middle quality control, HQC: High-quality control

Matrix samples stability at –28 ± 5°C for 37 days and –80 ± 5°C

Long-term stock solution stability for the prazosin was determined at a concentration of LQC-HQC level after a storage period of 37 days at −28°C and −80°C in refrigerator. The % mean stability of the prazosin was found to be 100.87% and 99.96% at 28 ± 5°C and 100.72% and 99.81% at 80 ± 5°C, respectively.

Long-term stock solution stability for the polythiazide was determined at a concentration of LQC-HQC level after a storage period of 37 days at −28°C and −80°C in refrigerator. The % mean stability of the polythiazide was found to be 100.41% and 100.01% at 28 ± 5°C and 100.11% and 99.98 at 80 ± 5°C, respectively.

Table 12: Precision and accuracy results for polythiazide

Acquisition batch ID	Date	HQC	MQC1	LQC	LLOQ QC
		Nominal concentration (ng/mL)			
		200.000	125.000	18.750	6.250
		Nominal concentration range (ng/mL)			
		(170.000–230.000)	(106.250–143.750)	(15.938–21.563)	(5.000–7.500)
		Back calculated concentration (ng/mL)			
		199.89	125.18	18.678	6.156
		200.15	125.00	18.462	6.258
		199.91	126.02	18.592	6.359
		198.93	125.09	18.188	6.062
		200.91	126.03	18.276	6.260
		201.82	125.02	18.568	6.161
n		6	6	6	6
Mean±SD		200.2677±0.99104	125.3880±0.49407	18.4607±0.19207	6.2093±0.10414
% CV		0.49	0.39	1.04	1.68
% mean accuracy		100.13	100.31	98.46	99.35
		201.89	125.20	18.501	6.260
		200.88	126.19	18.663	6.259
		200.92	125.18	17.920	6.574
		198.88	125.22	18.884	6.294
		196.90	126.16	18.669	6.262
		200.94	125.21	18.774	6.161
n		6	6	6	6
Mean±SD		200.0697±1.83772	125.5250±0.49984	18.5685±0.34232	6.3016±0.14092
% CV		0.92	0.40	1.84	2.24
% mean accuracy		100.03	100.42	99.03	100.83
		198.96	125.09	18.466	6.589
		200.95	125.17	18.592	6.157
		201.62	125.24	18.688	6.055
		201.24	126.20	18.476	6.205
		201.32	125.22	18.494	6.659
		200.59	126.24	18.389	6.225
n		6	6	6	6
Mean±SD		200.7770±0.95631	125.5282±0.53988	18.5175±0.10589	6.3151±0.24732
% CV		0.48	0.43	0.57	3.92
% mean accuracy		100.39	100.42	98.76	101.04
Between batch precision and accuracy					
n		18	18	18	18
Mean±SD		200.3714±1.28263	125.4804±0.48531	18.5156±0.22509	6.2753±0.17135
% CV		0.64	0.39	1.22	2.73
% mean accuracy		100.19	100.38	98.75	100.41

LLOQ: Lower limit of quantitation, LQC: Low-quality control, MQC: Middle quality control, HQC: High-quality control

RESULTS AND DISCUSSION

The method has been developed after performing several trails. In each trail, different columns, mobile phase, and flow rates were selected. The suitable wavelength for quantization was determined in K_2 EDTA human plasma and fixed chromatographic conditions, and the developed method is validated as per ICH guidelines; validated results are listed in Tables 3-15 and representative chromatograms present in Figures 1-11.

All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

Table 13: Recovery of prazosin

Acquisition batch ID						
Replicate No.	**HQC**		**MQC1**		**LQC**	
	Unextracted response	**Extracted response**	**Unextracted response**	**Extracted response**	**Unextracted response**	**Extracted response**
1	60,570	59,132	30,526	29,564	4530	4462
2	60,294	59,512	30,243	29,498	4509	4464
3	60,552	59,607	30,105	29,543	4486	4478
4	60,203	59,226	30,714	29,627	4529	4488
5	60,156	59,707	30,369	29,528	4523	4446
6	60,437	59,276	30,450	29,454	4509	4454
n	6	6	6	6	6	6
Mean±SD	60,369±177.24	59,410±230.88	30,401±214.29	29,536±58.90	4514±16.71	4465±15.42
% CV	0.29	0.39	0.70	0.20	0.37	0.35
% mean recovery	98.41		97.15		98.91	
Overall % mean recovery	98.160					
Overall SD	0.9074					
Overall % CV	0.92					

LQC: Low-quality control, MQC: Middle quality control, HQC: High-quality control

Table 14: Recovery of polythiazide

Acquisition batch ID						
Replicate No.	**HQC**		**MQC1**		**LQC**	
	Unextracted response	**Extracted response**	**Unextracted response**	**Extracted response**	**Unextracted response**	**Extracted response**
1	33,087	32,484	16,831	16,254	2518	2429
2	33,032	32,411	16,845	16,370	2520	2473
3	33,008	32,511	16,774	16,228	2527	2433
4	33,731	32,335	16,966	16,235	2536	2453
5	33,053	32,314	16,871	16,324	2542	2464
6	33,182	32,407	16,813	16,290	2520	2424
n	6	6	6	6	6	6
Mean±SD	33,182±275.62	32,410±78.14	16,850±65.46	16,284±55.62	2527±9.85	2446±20.22
% CV	0.83	0.24	0.39	0.34	0.39	0.83
% mean recovery	97.67		96.64		96.79	
Overall % mean recovery	97.033					
Overall SD	0.5598					
Overall % CV	0.58					

LQC: Low-quality control, MQC: Middle quality control, HQC: High-quality control

Table 15: Recovery – internal standard (celecoxib)

Acquisition batch ID	Date	
S. No.	Unextracted area ratio	Extracted area ratio
1	70,491	69,335
2	70,130	69,594
3	70,493	69,555
4	70,561	69,218
5	70,498	69,002
6	70,558	68,913
n	6	6
Mean±SD	70,455.2±162.52	69,269.5±280.14
% CV	0.23	0.40
% mean recovery	98.32	

The % CV for system suitability test was in the range of 0.89 for RT of prazosin , 1.15 for RT of polythiazide, and 0.74% for the area ratio (analyte area/IS area) of celecoxib.

Due to the auto-sampler was investigated by injecting a sequence of un-extracted and extracted samples. Results demonstrated that no significant carryover was observed during this experiment.

Matrix effect is played a key role in the assessment of pharmacokinetic studies. It was expressed as internal standard normalized matrix factor and it was varied from 0.90 to 0.99 which was close to 1 which indicates that there is no ionization suppression or enhancement in plasma samples.

QC samples

The chromatography observed during the course of prazosin and polythiazide which was acceptable and representative chromatograms of standard blank, standard zero (standard blank with internal standard) QC-LLOQ, QC-L, QC-M1, QC-M2, and QC-H samples sample are shown in Figures 3-9, respectively.

This indicates linearity of results and an excellent correlation between peak area ratios for each concentration of analytes.

The intraday and interday accuracy and precision of plasma samples were assessed and excellent mean % accuracy was obtained with range varied from 98.16 to 100.42% and 98.75 to 100.41% for intraday and 98.83 to 100.03 and 99.70 to 99.86 for interday, respectively. The precision (%CV) of the analytes and plasma samples was calculated and found to be 0.70–4.34% and 0.39–2.73% for intraday and 0.21–2.83% and 0.22–0.74% for interday, respectively. The results are summarized in Tables 11 and 12.

The overall % mean recovery for prazosin and polythiazide was found to be 98.16% and 98.23%.

The overall % mean recovery for celecoxib was found to be 98.11%.

CONCLUSION

Based on the results obtained in this study, it is concluded that the present validated method can be successfully applied for the estimation of prazosin and polythiazide in human plasma over the concentration range of 12.5–500 ng/m of prazosin and 6.25–250 ng/ml of polythiazide. The method for the determination of prazosin and polythiazide in human plasma using HPLC detection met the acceptance criteria with respect to selectivity, precision, accuracy, linearity, and recovery.

REFERENCES

1. Piascik MT, Perez DM. Alpha1-adrenergic receptors: New insights and directions. J Pharmacol Exp Ther 2001;298:403-10.
2. Madden CJ, Tupone D, Cano G, Morrison SF. α2 adrenergic receptor-mediated inhibition of thermogenesis. J Neurosci 2013;33:2017-28.
3. Giovannitti JA, Thoms SM, Crawford JJ. Alpha-2 adrenergic receptor agonists: A review of current clinical applications. Anesth Prog 2015;62:31-9.
4. Roush GC, Abdelfattah R, Song S, Ernst ME, Sica DA, Kostis JB. Hydrochlorothiazide vs chlorthalidone, indapamide, and potassium-sparing/hydrochlorothiazide diuretics for reducing left ventricular hypertrophy: A systematic review and meta-analysis. J Clin Hypertens (Greenwich) 2018;20:1507-15.
5. Eswarudu MM, Rao AL, Vijay E. Bioanalytical method development and validation for simultaneous determination of prazosin and polythiazide drugs in spiked human plasma by RP-HPLC. Int J Pharm Chem Biol Sci 2019;9:61-70.
6. Dokladalova J, Coco SJ, Lemke PR, Quercia GT, Korst JJ. Determination of polythiazide and prazosin in human plasma by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1981;224:33-41.
7. Sultana N, Arayne MS, Shah SN. Liquid chromatographic analysis of prazosin in API, dosage form and serum: Application to drug-metal interaction studies. J Chromatogr Sep Tech 2013;4:197.
8. Pawar VT, Pawar SV, More HN, Kulkarni AS, Gaikwad DT. RP-HPLC method for simultaneous estimation of cilnidipine and chlorthalidone. Res J Pharm Technol 2017;10:3990-6.
9. Sonawane LV, Poul BN, Usnale SV, Waghmare PV, Surwase LH. Bioanalytical method validation and its pharmaceutical application-a review. Pharm Anal Acta

2014;5:1-7.
10. Darkunde SL, Borhade RN. Bioanalytical method validation: A quality assurance auditor view point. Asian J Pharm Technol Innov 2017;5:59-60.
11. Tijare LK, Rangari NT, Mahajan UN. A review on bioanalytical method development and validation. Asian J Pharm Clin Res 2016;9:6-10.
12. Kumar A, Kishore L, Kaur N, Nair A. Method development and validation: Skills and tricks. Chron Young Sci 2012;3:3-11.
13. Latha EP, Sailaja B. Bioanalytical method development and validation by HPLC: A review. J Med Pharm Innov 2014;1:1-9.
14. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2 (R1). Current Step 4 Version, Complementary Guideline on Methodology. Geneva: ICH Expert Working Group; 1996.
15. ICH Expert Working Group. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Text and Methodology. Geneva: ICH Expert Working Group; 1996.

Source of Support: Nil. **Conflicts of Interest:** None declared.