

Development and Validation for the Simultaneous Quantification of Prazosin, Amlodipine, Diltiazem and Verapamil in API, Dosage Formulation and Human Serum by RP-HPLC: Application to *in vitro* Interaction Studies

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Abstract

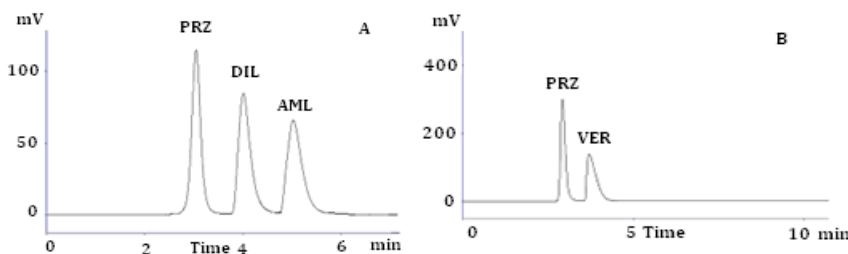
A novel reversed-phase liquid chromatographic method with UV detection for rapid and accurate simultaneous quantitation of prazosin (PRZ) and the key calcium channel blockers (CCBS), amlodipine besylate (AML), diltiazem hydrochloride (DIL) and verapamil hydrochloride (VER) in active pharmaceutical ingredients, pharmaceutical dosage formulations and human serum has been developed and validated according to ICH guidelines.

The reduced run time and low cost of analysis are additional merits of the method. This method showed the best resolution by using pre-packed Nucleosil® C₁₈ (10 μm, 25 × 0.46 cm) column at ambient temperature. The mobile phase consisting of methanol:water:acetonitrile (55:35:10 v/v; pH adjusted to 2.65 with phosphoric acid) was pumped at a flow rate of 1.0 mL min⁻¹ with an average operating pressure of 130 kg/cm² and effluent was monitored at 238 nm. Linearity of the method in the concentration range 5-100 μg mL⁻¹ for prazosin and 10-600 μg mL⁻¹ for calcium channel blockers showed good linear relationships for all the analytes (R² < 0.9998). The LLOD values were 32.8, 30.6, 54.2, 29.9 and LLOQ were 99.4, 92.6, 164.2, 90.5 ng mL⁻¹ for PRZ, AML, DIL and VER respectively, as per ICH guide lines acceptance criteria of 98 - 102%.

The newly developed method has been successfully employed for studying the interactions between prazosin and ca-channel blockers at simulated human body conditions; the results envisage a positive interaction between the two classes of drugs, as the percent recovery of the drugs almost changed, which indicate that prazosin may not be safe to co-administer with these antihypertensive drugs (Graphical Abstract).

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Graphical Abstract



PRZ prazosin; DIL Diltiazem; AML Amlodipine; VER Verapamil

Keywords: Prazosin; Amlodipine; Diltiazem; Verapamil; Human serum; Interaction studies

Introduction

Prazosin hydrochloride (PRZ) a 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl) piperazine (Figure 1a) [1] belongs to class of α₁ adrenergic blockers, which lowers the blood pressure by relaxing blood vessels. The central α₁-adrenergic mechanism is an important component in the regulation of hypothalamic corticotropin-releasing hormone secretion during stress [2] and effects on behavioral alteration induced by MK-801 in a spatial memory task [3,4] and has favorable effects on the plasma lipids, with reductions in total serum cholesterol [5]. PRZ can be used alone or in combination with thiazide diuretic and β- blocker in the treatment of hypertension [6] and can also be administered in chronic heart failure with ACE – inhibitors [7-9]. PRZ and CCBs are given in combination in the treatment of essential hypertension [10]. Boman et al. [11] have suggested that addition of PRZ may be of value in patients with severe heart failure

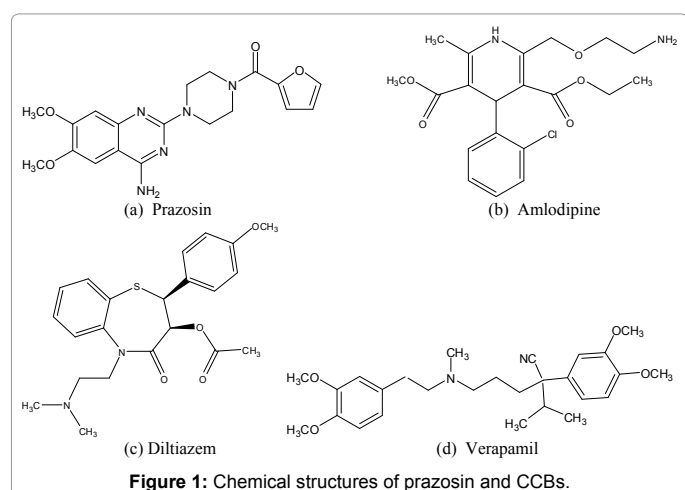
unresponsive to diuretics and digitalis combined with an ACE inhibitor, the combination of PRZ prazosin and an ACE inhibitor should theoretically reduce any activation of the rennin – angiotensin aldosterone system by prazosin.

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Calcium channel blockers (CCBs) are a structurally and functionally heterogeneous group of medications that are used widely to control blood pressure and manage symptoms of angina [12]. CCBs are particularly effective against large vessel stiffness, one of the common causes of elevated systolic blood pressure in elderly patients [13]. Amlodipine is dihydropyridine calcium channel blocker, often used to reduce systemic vascular resistance and arterial pressure, but are not used to treat angina, which carry an indication to treat chronic stable angina as well as vasospastic angina) because the vasodilation and hypotension can lead to reflex tachycardia. It can worsen proteinuria in patients with nephropathy [14]. Verapamil is phenylalkylamine calcium channel blocker, derived from phenethylamine, is relatively selective for myocardium, reduce myocardial oxygen demand and reverse coronary vasospasm, and is often used to treat angina. It has minimal vasodilatory effects compared with dihydropyridines and therefore causes less reflex tachycardia, which can be the most significant contributor to the heart's need for oxygen making it appealing for treatment of angina. Therefore, as vasodilation is minimal with the phenylalkylamines, the major mechanism of action is causing negative inotropy [15]. Caution should be taken when using verapamil with a β -blocker due to the risk of severe bradycardia [16]. Diltiazem a benzothiazepine calcium channel blocker belongs to the benzothiazepine class of compounds and is an intermediate class between phenylalkylamine and dihydropyridines in its selectivity for vascular calcium channels. It is administered orally in the treatment of angina pectoris and hypertension and may be given intravenously in the treatment of arterial fibrillation or flutter and paroxysmal supraventricular tachycardia [17]. The structures of CCBs used in this study are given in Figures 1b-d.

Literature survey has reported a number of analytical techniques for the determination of prazosin in biological fluids and pharmaceutical formulations by potentiometric titration [18], high performance liquid chromatography (HPLC) [19] and with fluorescence detector [20]. We have reported the quantitation of prazosin in active pharmaceutical ingredients (API), dosage formulations and serum and the method has been applied to study its interaction with metals [21]. Sahu and Patel [22] and Akram and El-Didamony [23] have reported the quantification of diltiazem, amlodipine and verapamil by ultraviolet (UV) spectrophotometry. High-performance liquid chromatographic (HPLC) method for the determination of diltiazem has been reported by Sultana et al. [24], whereas other workers have reported simultaneous determination of these Ca-channel blockers with other co-administered drugs [25-27]. Our research group earlier reported methods for the

Brand	Active Material	Drug (mg) per tablet	Manufacturer
Minipress	Prazosin	2 mg	Pfizer Pakistan Ltd
Norvasc	Amlodipine	5 mg	Pfizer Pakistan Ltd
Dilzem	Diltiazem	30 mg	Park-Davis & Company Ltd
Calan	Verapamil	40 mg	Searle Pakistan Ltd

Table 1: Details of API and dosage formulations

simultaneous determination of diltiazem and quinolones [28] and diltiazem with antidiabetic drugs [29] by HPLC.

Earlier we reported the simultaneous determination of prazosin with statins, in combined pharmaceutical dosage forms [30]. Literature reported that combination of CCBs and adrenergic receptor inhibitor could express more profound effects [31]. In present work, we developed a simultaneous HPLC method with UV detection for the quantitation of PRZ with AML, DIL and VER in commercial tablets and human serum, which has not been yet reported. The developed method is validated and has been used to study interactions between PRZ and CCBs (amlodipine, diltiazem and verapamil) for the routine monitoring of hypertensive patients who take a combination of these medications, and to study the pharmacokinetics of the combined dosage forms.

Experimental

Material and methods

The details API of prazosin and CCBs and their respective dosage forms are listed in Table 1. The API were kind gift from these manufacturers, while the dosage formulations were purchased from local pharmacies. They had an expiry of not less than one year at the time of study. All reagents used were of HPLC grade. Acetonitrile, methanol, and phosphoric acid 85% (E. Merck, Darmstadt, Germany) and HPLC-grade deionized water and working solutions were prepared daily. All solutions were filtered through a 0.45 μ m membrane filter (Millipore, Bedford, MA, USA) and degassed using a sonicator (Elma Germany).

Instrumentation

Two Shimadzu HPLC systems both from Shimadzu Corporation, Kyoto, Japan, one equipped with a liquid chromatography (LC)-10 AT VP pump and an SPD-10 A VP ultraviolet-visible detector, and another equipped with an LC-20AT and SPD-20A UV-VIS detector were utilized. Both the chromatographic systems were integrated via Shimadzu model CBM-102 to independent Intel Pentium IV computer (Intel Corporation, Santa Clara, CA, USA) loaded with Shimadzu CLASS-VP software (version 5.03, Shimadzu Corporation Japan) for data acquisition. A rheodyne manual injector (model 7725; Chrom Tech, Inc, Apple Valley, MN, USA) was fitted with a 20 μ L loop, Nuclisil[®] 100-10 C-18 column (250 mm \times 4.6 mm, 10 microns), and a DGU-14 AM online degasser (Shimadzu Corporation) were also used. In addition, electrical balance (Mettler Toledo # AB54), pH meter (Mettler Toledo MP 220), UV-Visible 1601 Shimadzu double beam spectrophotometer, 1 cm rectangular quartz cells, microliter syringe and micropore filtration assembly were used in this study.

Chromatographic conditions

The analysis was carried out isocratically with a mobile phase consisting of methanol:water:acetonitrile (55:35:10 v/v/v), with the pH adjusted to 2.65 \pm 0.02 using phosphoric acid (85%). The sample was injected through a 20 μ L loop and the mobile phase after filtration

through a 0.45 μm membrane filter was pumped at a flow rate of 1 ml min^{-1} . The effluent was monitored at 238 nm and analyses were carried out at ambient temperature ($\sim 25^\circ\text{C}$).

Preparation of standard and working solutions

Primary solutions of PRZ, AML, DIL & VER reference standards in 100, 300, 200 and 600 $\mu\text{g mL}^{-1}$ concentrations were prepared in a mixture of methanol and water (80:20) and appropriately diluted in concentration range of 5-100 $\mu\text{g mL}^{-1}$ of PRZ, 10-200 $\mu\text{g mL}^{-1}$ of DIL, 15-300 $\mu\text{g mL}^{-1}$ of AML, and 30-600 $\mu\text{g mL}^{-1}$ of VER in the same solvent. These solutions were then injected into the LC system ($n=6$).

Pharmaceutical formulations: 20 tablets of each pharmaceutical

formulation (Table 1) were accurately weighed and finely powdered. Amount of powder equivalent to 10 mg of PRZ, 20 mg of DIL, 30 mg of AML and 60 mg of VER were transferred individually to 100 mL volumetric flasks and dissolved by sonication with intermittent intervals for 60 minutes in methanol:water (80:20). Each flask was filled to mark and the resulting solutions were filtered through 0.45 μm filter. The aliquots were further diluted to get the desired concentrations.

Sample preparation for serum: Blood samples were collected from healthy volunteers (who consent was already sought and none of those were on medication, smoking or in any other strenuous activity) in evacuated glass tubes through an indwelling cannula placed on forearm vein by a trained clinical laboratory technician (Fatmid Foundation Hospital, Karachi, Pakistan). The blood was shaken with acetonitrile

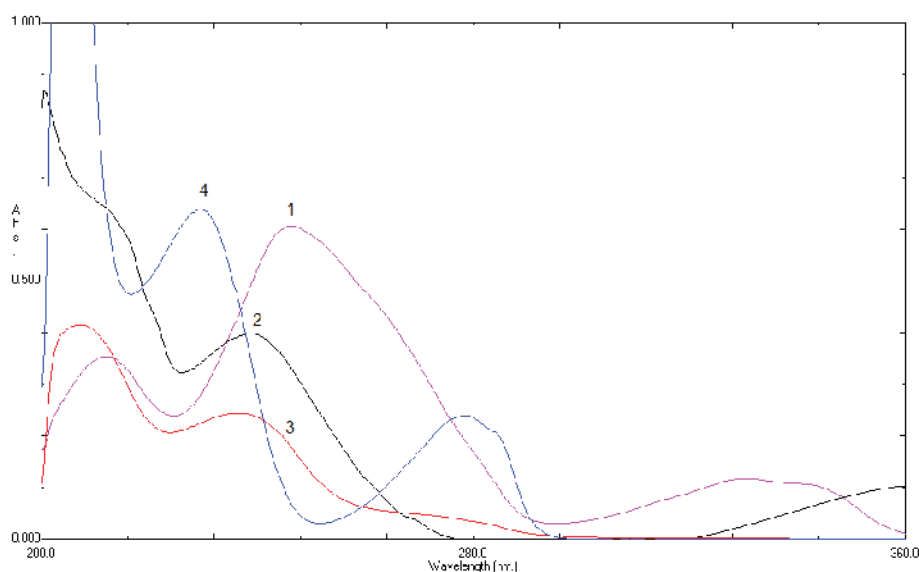


Figure 2: UV spectrum of 1=prazosin, 2=amlodipine, 3=diltiazem and 4=verapamil.

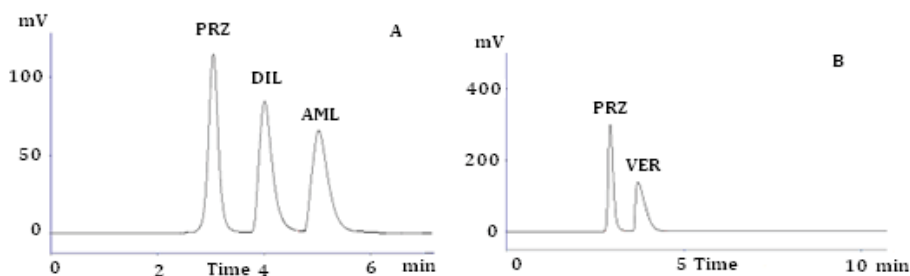


Figure 3: Chromatogram of prazosin, diltiazem and amlodipine (A); prazosin and verapamil (B) in formulation.

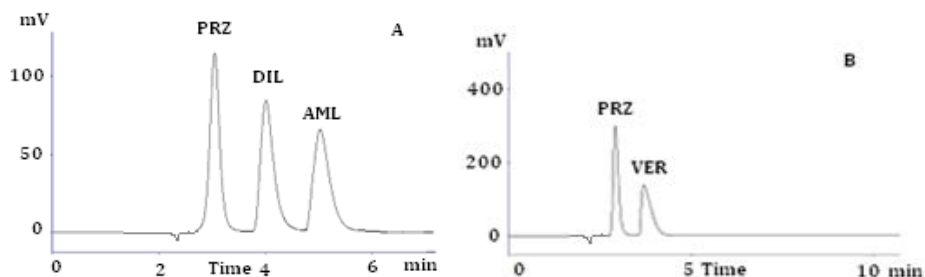


Figure 4: Chromatogram of prazosin, diltiazem and amlodipine (A); prazosin and verapamil (B) in human serum

Drugs	Concentration ($\mu\text{g mL}^{-1}$)	Regression equation	r^2	LLOD ng mL^{-1}	LLOQ ng mL^{-1}
Prazosine	5-100	$y = 29490x + 29699$	0.9999	32.8	99.4
Amlodipine	15-300	$y = 27735x + 18635$	0.9998	30.6	92.6
Diltiazem	10-200	$y = 29072x + 17699$	0.9999	54.2	164.2
Verapamil	30-600	$y = 26185x + 39843$	0.9999	29.9	90.5

Table 2: Regression parameters

Analyte	Spiked conc.	In bulk		In serum	
	($\mu\text{g mL}^{-1}$)	% Rec.	% RSD	% Rec.	% RSD
Prazosin	8	100.08	0.009	100.04	0.004
	10	99.93	0.004	99.97	0.002
	12	100.65	0.001	100.07	0.008
Amlodipine	32	100.21	0.008	100	0.018
	40	100.09	0.085	100.03	0.008
	48	99.55	0.002	99.98	0.001
Diltiazem	16	100.17	0.007	100.06	0.011
	20	100.09	0.005	99.96	0.005
	24	100.1	0.001	100.03	0.002
Verapamil	48	100.05	0.002	100.05	0.004
	60	99.94	0.003	99.92	0.004
	72	99.98	0.054	100.09	0.017

Table 3: Accuracy of the proposed method

Conc. $\mu\text{g mL}^{-1}$	Day 1 (% Recovery)				Day 2 (% Recovery)				Day 3 (% Recovery)			
	PRZ	AML	DIL	VER	PRZ	AML	DIL	VER	PRZ	AML	DIL	VER
API												
5	99.28	99.79	100.2	100.0	99.98	99.25	101.2	100.5	99.52	99.82	100.5	100.3
10	99.93	100.1	100.1	99.94	99.85	98.99	100.2	99.24	99.74	99.51	99.58	98.83
15	98.95	100.0	99.81	99.58	100.2	98.74	100.5	100.7	99.94	99.29	100.1	100.2
20	99.71	99.60	98.59	99.90	99.65	99.68	99.87	99.83	99.28	100.7	99.58	99.57
25	100.2	100.5	99.96	100.3	98.59	99.89	100.7	99.17	99.51	99.59	101.2	98.46
50	100.7	99.28	100.2	100.6	100.2	100.1	100.0	100.5	100.0	99.87	99.51	100.1
100	99.97	98.89	100.1	99.08	99.87	99.05	99.96	100.9	99.57	99.26	99.27	99.87
Serum												
5	100.0	99.28	99.86	99.95	100.2	99.16	100.8	100.2	99.01	99.51	100.2	100.1
10	99.65	99.58	100.0	100.1	100.5	98.58	100.4	9.65	99.97	99.01	100.7	99.28
15	99.18	99.71	99.67	100.8	100.8	98.11	100.2	100.4	98.28	99.64	99.96	99.17
20	99.50	99.83	99.58	99.92	99.85	99.19	100.1	99.92	99.87	98.17	99.57	99.51
25	99.68	100.1	100.0	100.2	99.69	99.38	100.9	99.75	100.2	99.84	101.0	100.1
50	99.98	99.05	99.68	100.6	99.53	99.82	99.58	100.1	99.17	99.63	99.15	99.47
100	99.37	99.18	101.0	99.87	99.08	98.95	100.2	100.4	98.96	99.50	100.0	99.96

Table 4: Intra-and inter day, variation /intermediate precision of the method

Parameters	Variation	% RSD			
		PRZ	AML	DIL	VER
Chromatographic Condition					
Mobile phase composition	MeOH:H ₂ O:ACN (50:40:10)	0.047	0.011	0.015	0.057
	MeOH:H ₂ O:CAN (60:30:10)	0.01	0.017	0.019	0.021
pH of mobile phase	2.63	0.745	0.042	0.054	0.145
	2.67	0.014	0.019	0.014	0.021
Flow rate (mL min^{-1})	0.9	0.241	0.032	0.061	0.01
	1.1	0.417	0.421	0.019	0.005
Column temperature ($^{\circ}\text{C}$)	22	0.528	0.247	0.013	0.007
	30	0.015	0.087	0.041	0.018
Detection wavelength (nm)	238	0.065	0.024	0.081	0.045
	242	0.021	0.004	0.014	0.085

Table 5: Robustness of the method

(10:90, v/v) and centrifuged at 10,000 rpm for 10 min to deprotonate it. The serum thus obtained was filtered and used for the analysis [32]. Stock standard solutions were spiked with serum to prepare desired working solutions and analyzed. These serum solutions were stored at -20°C for pending analysis and for inter day variation studies.

Sample preparation for interaction studies

Stock solutions of PRZ, DIL, AML and VER in $100 \mu\text{g mL}^{-1}$

concentration were individually prepared in buffers of pH 1, 4, 7.4 and 9 to simulate human GI tract. PRZ was then mixed with each of calcium channel blockers separately in 1:1 ratio in Erlenmeyer flasks, which gave the final concentration of $50 \mu\text{g mL}^{-1}$ for each constituent. The flasks were maintained at 37°C for three hours with frequent shaking. Aliquots of 2 ml were withdrawn at 30 minute intervals for 180 minutes, appropriately diluted, filtered (through Millipore filter $0.45 \mu\text{M}$) and chromatographed.

Drugs	System	Concentration ($\mu\text{g mL}^{-1}$)		Recovery (%)
		Added	Found	
PRZ	LC 10	8	8.01	100.08
		10	9.99	99.93
		12	12.08	100.65
	LC 20	8	7.99	99.85
		10	10	99.99
		12	12.1	100.85
AML	LC 10	32	32.07	100.21
		40	40.04	100.09
		48	47.78	99.55
	LC 20	32	32.17	100.54
		40	40.44	101.1
		48	48	100.01
DIL	LC 10	16	16.03	100.17
		20	20.02	100.09
		24	24.02	100.1
	LC 20	16	15.88	99.28
		20	20.05	100.26
		24	24.35	101.45
VER	LC 10	48	48.02	100.05
		60	59.96	99.94
		72	71.99	99.98
	LC 20	48	48.67	101.4
		60	60.02	100.04
		72	72.34	100.47

Table 6: Ruggedness of the proposed method

Results and Discussion

The method developed for the simultaneous quantitation of prazosin with calcium channel blockers is simple, precise, accurate and sensitive. Chromatographic conditions were optimized to achieve best separation and to get best resolution between analytes and to optimize chromatographic parameters like resolution, tailing factor and retention time. For validation of analytical methods, the ICH guidelines [33] and USP 2007 [34] were followed for the specificity, accuracy tests, precision, linearity, work strip and robustness of the method.

Method development and optimization

Some chromatographic parameters such as column type, mobile phase, wavelength and conditioning time were investigated to obtain a suitable peak for all analytes within an acceptable time and limit. In order to optimize column packing, C_8 and C_{18} columns with different lengths and particle sizes were used. Nuclosil, C_{18} (250×4.6 mm, 10 μm) column provided an efficient and reproducible peak area with sharp peaks at ambient temperature. Different combinations and ratios of methanol, water and acetonitrile were tried for mobile phase but the best composition, which delivered optimum results was achieved with methanol, water and acetonitrile in the ratio of 55:35:10 (v/v/v). However, the pH of this mobile phase was to be adjusted to 2.65 ± 0.02 with phosphoric acid to have good resolution without tailing or fronting. Variation in pH of the mobile phase also had a great influence to the retention time and peak sharpness of analytes. If percentage of methanol in the mobile phase was increased the retention time of CCBs decreased and the peaks merged with prazosin. The optimum wavelength selected for analysis was on the basis of UV scan of all interacting drugs, which showed maximal absorbance near 238 nm (Figure 2).

System suitability testing: An integral part of chromatographic methods, system suitability test was used to verify the resolution and

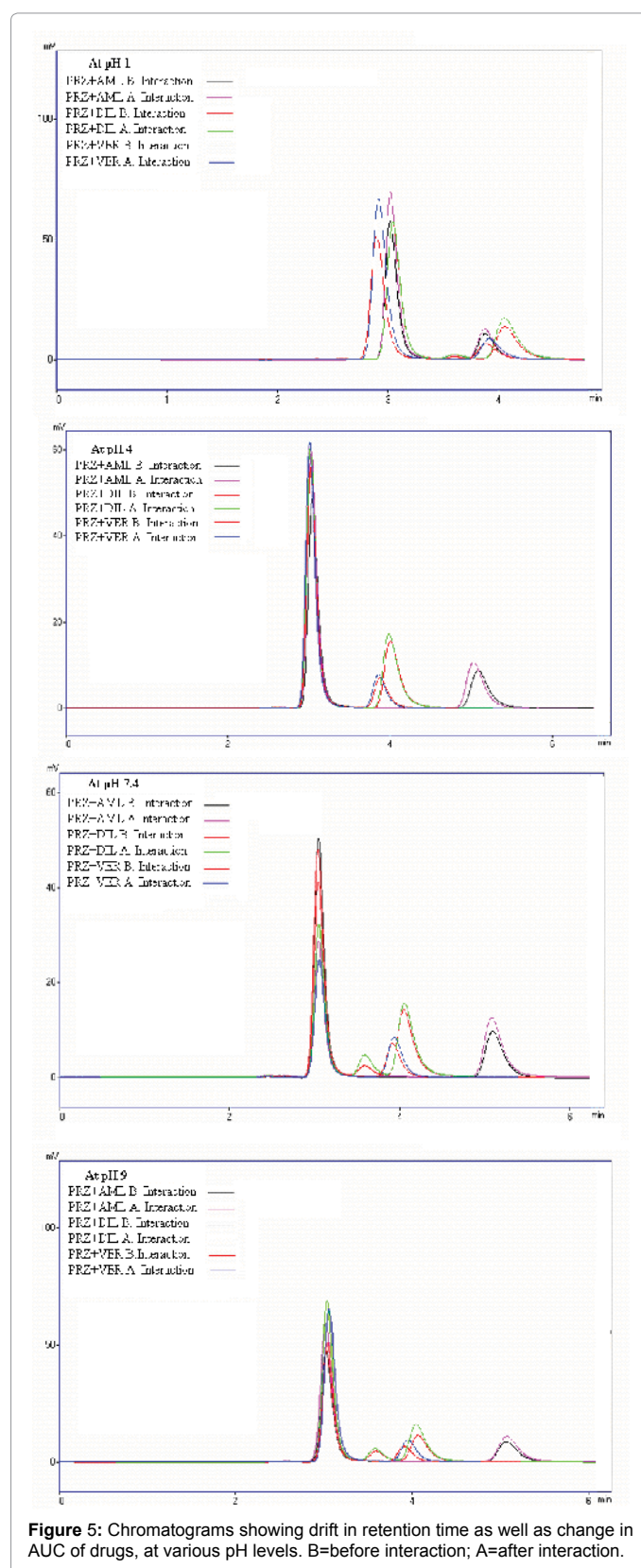


Figure 5: Chromatograms showing drift in retention time as well as change in AUC of drugs, at various pH levels. B=before interaction; A=after interaction.

reproducibility of the system for adequate analysis. The HPLC system was equilibrated with the mobile phase, followed by six injections of the standard. These consecutive injections were used to evaluate the system suitability on each day of method validation. All peaks were

well resolved and the precision was acceptable. Parameter of system suitability was peak symmetry (symmetry factor), theoretical plates of the column, mass distribution ratio (capacity factor) and tailing factor.

Specificity and selectivity: Selectivity and specificity of the chromatographic method was determined to ensure the separation of prazosin and CCBs. The method demonstrated fine resolutions and was found to be free of interference from excipients (Figure 3) used in dosage formulations; thus the method was specific for prazosin and CCBs (Figure 4) in serum as well. These chromatograms showed no additional peaks due to excipients or endogenous serum components at the same retention time of analyte, and hence the specificity was confirmed.

Range and linearity: Linearity is generally reported as the variance of the slope of the regression line. It was tested in order to demonstrate a proportional relationship of response versus analyte concentration. It was studied at seven concentration levels in the range of 5-100 $\mu\text{g mL}^{-1}$ for prazosin; 15-300 $\mu\text{g mL}^{-1}$ for amlodipine, 10-200 $\mu\text{g mL}^{-1}$ for diltiazem and 30-600 $\mu\text{g mL}^{-1}$ for verapamil. Six replicates were performed for every concentration. Injected concentrations versus peak area were plotted, and the correlation coefficients were calculated, which are given in Table 2.

Accuracy and recovery studies: The accuracy of the procedure which gauge the closeness of measured values to the true values was evaluated as percentage relative error between the measured mean concentrations and taken concentrations. A minimum of 3 concentration levels covering the specified ranges were selected and six

runs were performed for every concentration and then % recovery was calculated (Table 3).

Precision: The intra- and inter-day variability or precision was evaluated by assaying the sample's seven concentrations per day for three consecutive days (Table 4). In this assay, the intra-day precision and the inter-day precision recovery was 98–102%. The % RSD was found to be less than 2%, which indicated that the method was sufficiently precise and accurate.

Limits of detection and quantitation: The ratio of chromatographic signal when remained three times and ten times with their respective noise signals was concluded for LLOD and LLOQ respectively. The LLOD and LLOQ for PRZ, AML, DIL and VER were 32.8, 30.6, 54.2 and 29.9 ng mL^{-1} and 99.4, 92.6, 164.2 and 90.5 ng mL^{-1} respectively in bulk material (Table 2).

Robustness: Robustness of the method was accomplished by design modifications made to the method parameters such as composition of mobile phase, flow rate, pH of the mobile phase, detection wavelength, and column temperature (Table 5), and it was found that the percentage of RSD values did not exceed more than 2%.

Ruggedness: The ruggedness of the study was established by determining PRZ, AML, DIL, and VER using two different chromatographic systems LC10 and LC20 and same column. The assay results indicated that the method was equally capable with high precision (Table 6) on both the instruments.

Time (mins)	pH 1		pH 4		pH 7.4		pH 9	
	Prazosin and amlodipine (available %)							
	PRZ	AML	PRZ	AML	PRZ	AML	PRZ	AML
0	99.89	98.78	99.59	99.48	100.15	100.28	100.29	98.48
30	92.19	93.72	100.19	99.12	105.00	102.93	104.58	97.74
60	93.75	95.30	107.24	106.57	104.90	104.97	100.77	101.99
90	98.67	100.34	110.08	109.81	79.35	107.29	110.85	111.23
120	108.21	110.79	114.40	110.73	62.70	112.19	118.26	119.53
150	114.94	116.23	116.43	110.40	63.61	120.26	121.90	119.07
180	119.59	122.08	119.40	117.34	55.44	127.20	131.78	130.38
	Prazosin and Diltiazem (available %)							
	PRZ	DIL	PRZ	DIL	PRZ	DIL	PRZ	DIL
0	101.21	100.85	100.10	99.98	100.12	100.25	100.28	100.59
30	104.26	105.04	101.59	100.38	104.55	104.09	101.12	101.79
60	107.37	107.07	101.97	101.58	77.84	106.42	100.43	98.47
90	108.84	108.91	100.70	101.79	79.79	106.38	111.24	113.06
120	109.99	109.94	101.51	102.33	48.75	106.33	117.43	124.39
150	114.86	115.81	110.14	109.98	52.74	106.41	119.63	123.68
180	116.93	117.08	110.68	110.76	61.24	117.30	133.12	141.07
	Prazosin and Verapamil (available %)							
	PRZ	VER	PRZ	VER	PRZ	VER	PRZ	VER
0	100.54	101.12	99.89	100.21	99.87	99.78	100.01	100.12
30	122.85	115.74	99.99	101.01	98.54	99.42	102.57	102.72
60	120.98	112.95	102.25	103.09	96.63	101.47	108.06	105.06
90	129.20	120.11	102.67	103.74	77.00	102.85	115.62	115.91
120	139.38	131.47	107.34	109.61	61.78	107.80	117.44	117.14
150	145.90	134.07	107.61	108.47	61.06	109.65	130.29	131.88
180	158.10	150.22	108.61	111.03	50.86	117.27	142.63	143.29

Table 7: Interaction studies between prazosin and calcium channel blockers at 37°C

Interaction studies using RP-HPLC

Results of interactions studies of prazosin with calcium channel blockers are summarized in Table 7. The recovery of PRZ increased to 120–132 % in presence of AML in simulated gastric juice, buffers of pH 4 and 9, while in buffer of pH 7.4 the recovery of PRZ first increased to 105 % and later decreased to 55 % at the end of the experiment. On the other hand, the recovery of AML increased up to 122–130% in all interacting mediums. In case of both DIL and VER the recovery of prazosin increased in all buffers, whereas the recovery of PRZ in pH 7.4 decreased to 61 and 51% respectively at the end of the experiment. Similar behavior was observed in case of VER, the recovery increased in all buffers. Conclusively, a remarkable change in AUC and drift in retention time (Figure 5) are evident of the interactions of PRZ with CCBs under study. Hence, it would not be safe to co-administer prazosin with these interacting drugs. These two classes of drugs inhibit or disturb the absorption of each other.

Conclusion

High performance liquid chromatographic method for the simultaneous quantitation of prazosin and calcium channel blockers (amlodipine, diltiazem and verapamil) was developed and validated for the first time. The current method is very simple, fast and easy to perform, shows a high percentage of precision and recoveries, shorter run time, has low limits of detection and quantification values up to nanogram levels and is linear up to a wide range of concentrations. This makes the method applicable for routine clinical analysis, multidrug pharmacokinetic study and also in the study of drug-drug interactions.

The developed method has been applied to the monitoring of *in vitro* interactions of PRZ with these CCBs. In the light of above results, it is inferred that certain medicines can interact pharmacologically and affect the activity of others if they are mixed during their administration. Hence the concurrent administration of these drugs may affect the bioavailability of PRZ, which may result in synergistic or antagonistic therapeutic effect.

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