Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

SIMULTANEOUS DETERMINATION OF TWO ALPHA-ONE ADRENORECEPTOR BLOCKERS TERAZOSIN AND PRAZOSIN USING TAMSULOSIN AS INTERNAL STANDARD

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Received: 29 May 2012, Revised and Accepted: 19 Jun 2012

ABSTRACT

In the presented study simultaneous determination of prazosin and terazosin was performed using another alpha one blocker tamsulosin hydrochloride as internal standard. Method was developed using Waters HPLC system equipped with the Empower software and composed of Quadrupole 600E gradient pump equipped with Kromasil C18 column (250 × 4.6 mm, 5.0 µm) from Agilent Technologies using methanol as mobile phase. Method was than validated in terms of specificity, accuracy, linearity, precision, limit of detection, limit of quantitation and system suitability. Developed method was successfully applied for the determination of Terazosin and Prazosin in tablet pharmaceutical.

Keywords: Terazosin, Prazosin, Method development, Validation, Tamsulosin, Internal standard.

INTRODUCTION

Benign prostatic hyperplasia (BPH), a condition characterized by hyperplastic nodules in the periurethral region and transition zone of the prostate, overall prostatic enlargement, and lower urinary tract symptoms, is highly prevalent among middle-aged and elderly men. BPH is rarely life-threatening, but it can lead to acute urological problems, for example acute urinary retention (AUR). α_1-Adrenoceptors belong to the superfamily of G-protein coupled adrenergic receptors which mediate actions of endogenous catecholamines (norepinephrine and epinephrine). α_1-blockers reduce smooth muscle tone in the prostate and result in rapid improvements in urinary symptoms and flow. Currently available α_1-blockers include the nonselective α_1-blockers, terazosin, doxazosin, and alfuzosin, and the highly selective α_{1a}-blocker, tamsulosin. These agents have comparable efficacy; the main difference among these agents relates to their tolerability profiles. An extensive review of data from trials of α_1-blockers, including data from 6333 patients in placebo-controlled trials and 507 patients in direct comparative studies, revealed that alfuzosin (sustained- release formulation) and tamsulosin (0.4 mg modified-release formulation) are better tolerated than terazosin and doxazosin[1].

Prazosin 1-4 (amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanyl carbonyl) piperazine HCl[2] is official in British Pharmacopeia[3], Indian Pharmacopoeia[4], United States Pharmacopoeia[5] and European Pharmacopoeia[6]. Two spectrophotometry[7,8], six chromatography[9-14], potentiometry [3,4,15] voltametric [8] and radio receptor assay [16] related to the determination of prazosin found in the literature.

Terazosin hydrochloride dehydrate RS-1-(4-amino-6, 7-dimethoxy-2-quinazolinyl)-4-[(tetra-hydro-2-furanyl) carbonyl] -piperazine monohydrochloride is official in Europian Pharmacopoeia [6]. Two spectrophotometric methods [17,18], two TLC[15] and HPLC methods [19-22] including two HPLC method recommended by European Pharmacopoeia[6] and potentiometry[15,6] are reported methods.

Simultaneous determination of prazosin, terazosin and doxazosin[23] is also available with current literature. Another published literature is simultaneous determination of prazosin, terazosin, doxazosin, tamsulosin and alfuzosin in their respective phramceutical dosage forms[24] by using HPLC and HPTLC.

The analytical chemistry hence has challenge in developing the methods for their analysis with the help of number of analytical techniques, which are available for the estimation of the drugs and their combination. Analytical monitoring of pharmaceutical product is necessary to ensure the efficacy and safety throughout the shelf life, including storage, distribution and use[25]. Most of the major developments in analytical chemistry take place after 1900. During this period instrumental analysis becomes progressively dominant in the field[26].

HPLC was the dominant separation technique in modern pharmaceutical and biomedical analysis, because it resulted in highly efficient separations and in most cases provided high detection sensitivity. Chromatographic optimization procedures are becoming more multidisciplinary, as methods are sought to obtain a lot more information on the separations, which may be isocratic or gradient[27]. Similar molecular structures are difficult to analyze simultaneously as shown under Figure 1, both terazosin and prazosin have quinazoline nucleus in their structure.

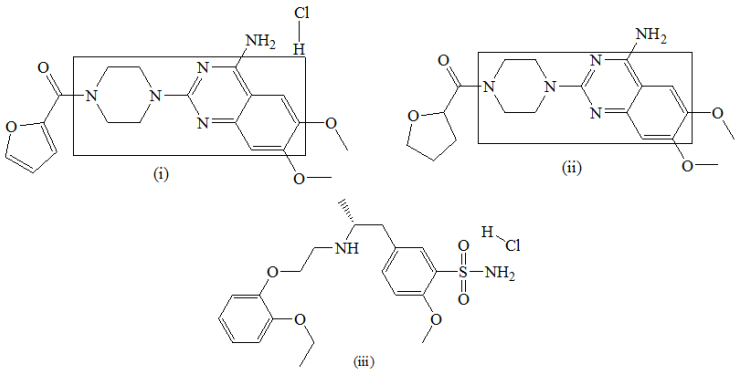


Fig. 1: Chemical structure of (i) Prazosin hydrochloride (ii) Terazosin (iii) Tamsulosin hydrochloride (Internal Standard) [Quinazoline nucleus in boxes]

As a result of technology developments during the past decade which have brought significant improvements to instrumentation and column packings, high performance liquid chromatography (HPLC) has emerged as the preferred method for the separation and quantitative analysis of wide range of samples[28]. Thus the aim of the presented work is to develop simple, accurate and precise HPLC method for simultaneous determination of TRZ and PRZ in pharmaceutical dosage forms.

MATERIAL AND METHOD

Prazosin and Terazosin were obtained from Arti laboratories and Nosch Pharma respectively. Methanol and water (HPLC-grade) were purchased from Merck Ltd (Mumbai). Tablet formulations, Terapress 2 mg batch no. DM1160 (Intas Pharmaceuticals) and Prazopress XL 5 mg batch no. JKK1147 (Sun Pharmaceuticals) were purchased from local market.

Waters HPLC system equipped with the Empower software and composed of Quadrupole 600E gradient pump with four channel multisolvent delivery system, an online Waters In-line degasser AF, a column oven model HCO-O2 (PCI analyst) and a 2998 PDA detector. All separations were carried out on a Kromasil C18 column (250 × 4.6 mm, 5.0 µm) from Agilent Technologies is used for method development.

Selection of wavelength

Wavelength selection is based on UV absorbance of TRZ, PRZ and TAM. It was found that 230 nm will be most suitable wavelength to proceed presented study.

Optimization of mobile phase

Mobile phase was optimized using methanol alone runned isocratically at flow rate of 1.1 ml/min. Good symmetrical peaks were observed for PRZ, TRZ and internal standard TAM. The retention time of TRZ, PRZ and TAM found to be 7.924, 11.414 and 1.821 minutes respectively. Tailing factors found are 1.06 and 1.18 respectively for TRZ and PRZ. Likewise capacity factors for TRZ and PRZ found to be 6.9 and 10.4 respectively.

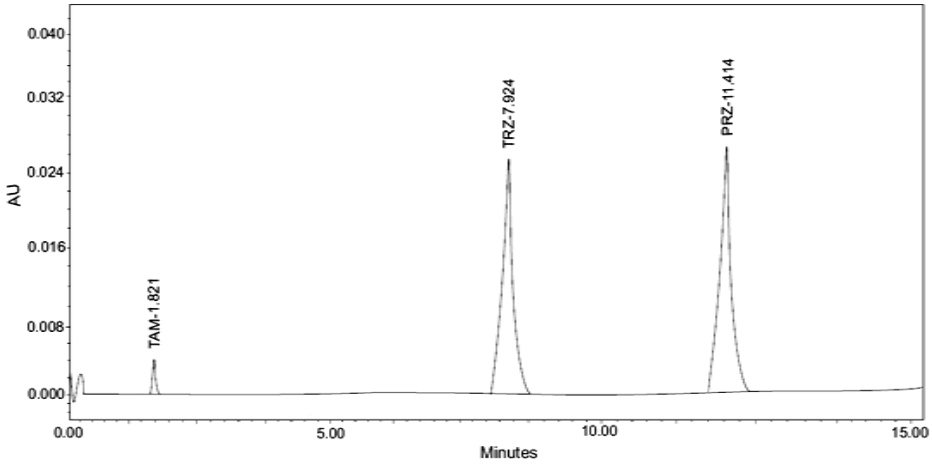


Fig. 2: Optimized chromatogram condition of the proposed method.

Preparation of calibration curve

Methanol was used to prepare dilution for calibration curve containing 5 µg/ml of TAM. Suitable aliquots of standard stock solution (containing 1000 µg/ml of TRZ and PRZ and 5 µg/ml TAM) 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml were transferred to 10 ml volumetric flasks and diluted with methanol containing internal standard to obtain series of solutions of 10, 20, 30, 40, 50 and 60 µg/ml respectively. The resulting solutions were than filtered from 0.45 µ filter and used as such for preparation of calibration curve. Same procedure was repeated three times.

Calibration curve was plotted between concentration and ratio of areas of TRZ and PRZ with TAM (internal standard). Regression equation and correlation coefficients are calculated.

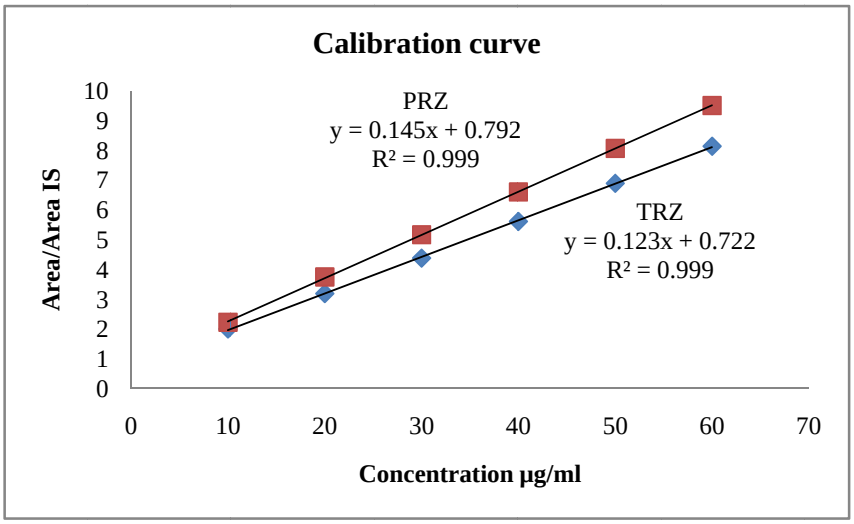


Fig. 3: Calibration curve of TRZ and PRZ

Validation of proposed method

Specificity

Specificity of the proposed method was established by spiking synthetic mixture of the drugs with commonly used excipients such as starch, lactose, magnesium stearate, titanium dioxide in proportions generally used in the tablets. Blank was also prepared by spiking excipients with methanol containing only internal standard. One more blank was prepared by spiking excipients with methanol (without internal standard).

This is clear from chromatogram (Figure 4) that there is no interference of excipients near to the retention time of any of the peak.

Linearity

Linearity was assessed by visualizing calibration curve and it was found that all of the points are near to the regression line and distributed on both sides.

Precision and stability

Repeatability was assessed by injecting six replicates of same concentration (20 µg/ml). Standard deviation between peak area observed and % relative standard deviation (% RSD) were calculated.

% RSD less than one proves method's repeatability.

Intraday: 0.2, 0.3 and 0.4 ml from the standard stock solution were diluted to 10 ml with methanol to produce 20, 30 and 40 µg/ml solution. Solutions were injected three times in a day.

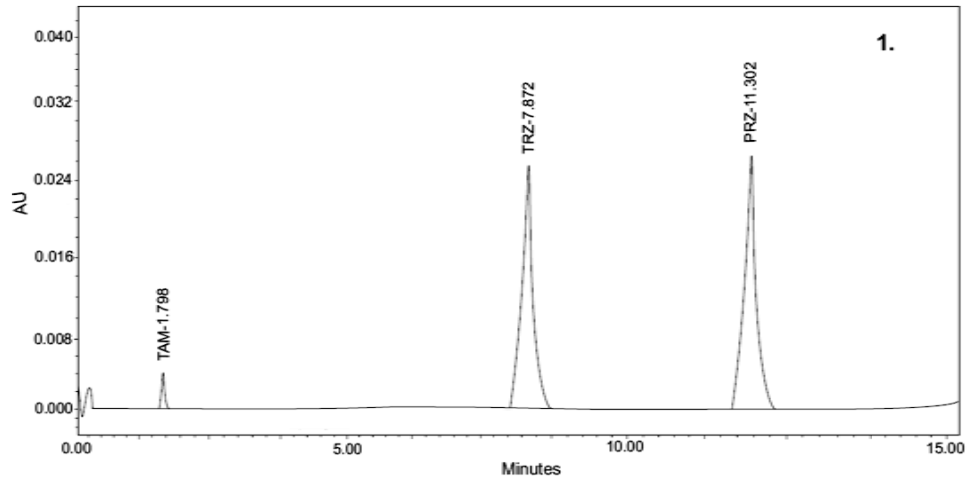
Interday: Same procedure was followed as mentioned in previous paragraph and solutions were injected in three consecutive days.

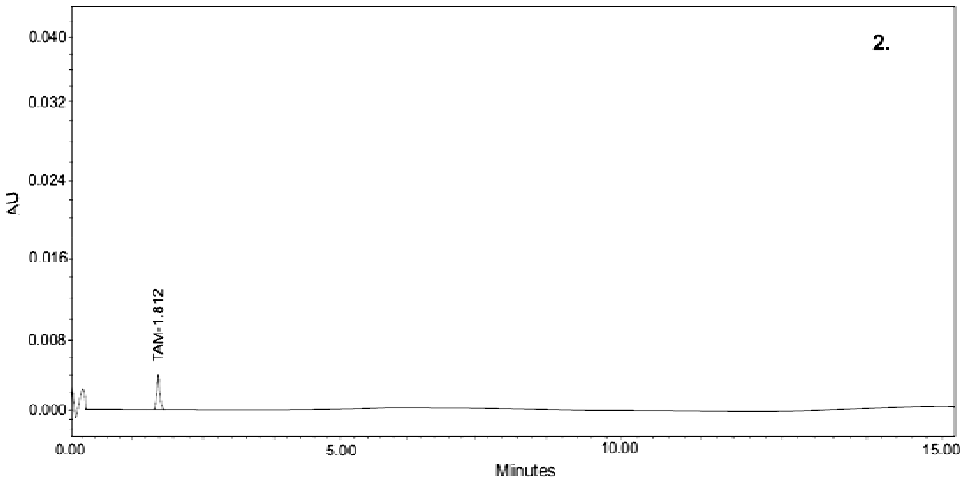
% RSD in peak area for interday and intraday precision found to be less than 2 %. In this way method was found to be sufficiently precise in nature.

For stability test, a sample solution was analyzed every 12 h for 2 days, and the sample solution was found stable within 48 h (R.S.D. < 2.0%)

Accuracy

Dilutions of 20, 30 and 40 µg/ml were prepared by standard stock solution and spiked with solution containing solution of 10 µg/ml concentrations of both TRZ and PRZ. Percentage recovery obtained is mentioned under Table 1 and 2.





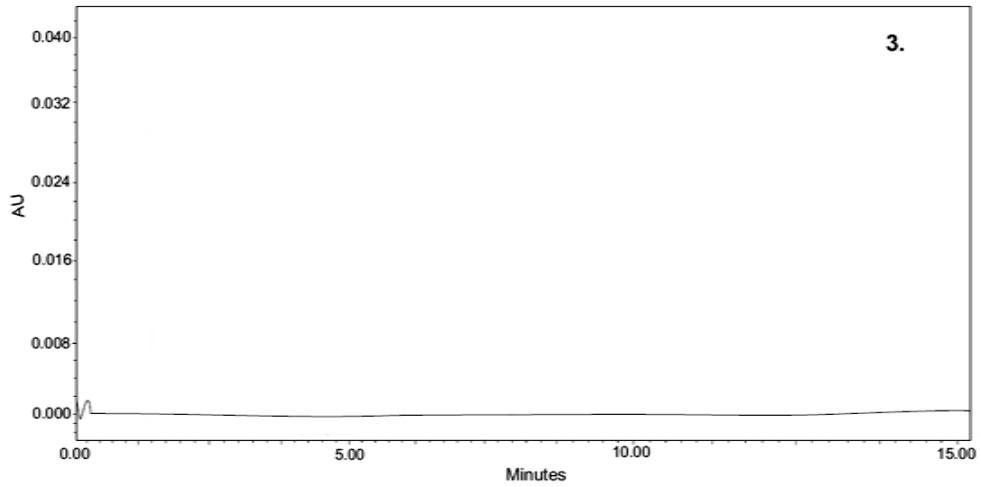


Fig. 4: Chromatograms 1. TRZ and PRZ with excipients 2. TAM (Internal standard) with excipients 3. Excipients alone.

Fig. 4: Chromatograms (1) TRZ and PRZ with excipients (2) TAM (Internal standard) with excipients (3) Excipients alone.

Table 1: Recovery studies for TRZ in Terapress tab (Intas)

Concentration found (TRZ) in µg/ml			Concentration found after spiking in µg/ml			% Recovery			Mean
20	**30**	**40**	**10**	**20**	**30**	**10**	**20**	**30**	
20.1	29.7	40.1	30.2	39.7	50	100.49	100	99.75	99.90±0.470
20	29.8	40.1	29.8	39.9	50.1	99	100.33	100	
20	29.7	40	29.9	39.8	49.9	99.5	100.33	99.75	

Table 2: Recovery studies for PRZ in Prazopress XL tab ((Sun Pharmaceuticals)

Concentration found (PRZ) in µg/ml			Concentration found after spiking in µg/ml			% Recovery			Mean
20	**30**	**40**	**10**	**20**	**30**	**10**	**20**	**30**	
20	30.1	39.9	29.8	39.8	49.8	99	99.0	99.7	99.43±0.456
20.1	29.9	39.8	29.9	39.8	49.9	99	99.6	100.2	
20	30	39.8	29.9	39.7	49.7	99.5	99	99.7	

Overall % recoveries found to be between 98-102% proves method accurate.

Limit of detection and limit of quantitation[29]

Limit of detection (LOD) and limit of quantification (LOQ) were calculated through standard deviation of the response of calibration curve by using following formula

$$LOD = 3S_a/b \qquad LOQ = 10S_a/b$$

Where, S_a is the standard deviation of the response and b is the slope of the calibration curve.

LOD and LOQ for TRZ found was 0.514 and 1.557 µg/ml and for PRZ 0.511 and 1.549 µg/ml respectively.

System suitability testing

System suitability testing was performed by using six replicates of test concentrations. Variations in Tailing factor, RT, and theoretical plates (N) were calculated as average of six replicates.

Sample analysis

Both methods were then applied in pharmaceutical formulations purchased from local pharmacy market. Results found are presented under Table 4.

Table 3: Results of system suitability test of proposed method.

	Tailing Factor		No. of theoretical plates		Retention time	
	TRZ	**PRZ**	**TRZ**	**PRZ**	**TRZ**	**PRZ**
	1.06	1.17	8564	9865	7.934	11.31
	1.05	1.18	8454	9833	7.978	11.21
	1.07	1.19	8512	9877	7.98	11.234
	1.07	1.18	8534	9867	7.886	11.222
	1.06	1.17	8544	9855	7.897	11.467
	1.05	1.18	8524	9884	7.888	11.366
Mean	1.06	1.178333	8522	9863.5	7.927167	11.3015
SD	0.008944	0.007528	37.73592	17.99722	0.043728	0.100868
RSD	0.843799	0.638845	0.442806	0.182463	0.551626	0.892516

Table 4: Result found from analysis of marketed formulations

Name of formulation	Drug	Concentration found (mg)	Mean	SD	RSD
Terapress (Intas)	Terazosin	1.98	1.97	0.00577	0.292
		1.97			
		1.97			
Prazopress (Sun Pharma)	Prazosin	5.01	5.02	0.01154	0.229
		5.03			
		5.03			

CONCLUSION

In this way method for simultaneous determination of terazosin and prazosin in marketed formulation was developed and validated. Method was validated in terms of specificity, linearity, precision, LOD, LOQ, accuracy and system suitability. Method was found to be specific in nature as there was no interference of commonly used excipients near or at the retention time of the drugs. Method was also found to be sufficient precise and accurate to be utilized in pharmaceutical industries. Developed method is simple enough and does not requires skilled personnel to be used for determination of these drugs.

ACKNOWLEDGEMENT

Authors wish to acknowledge support given by TIFAC-CORE grant given by department of science and technology, Government of India for purchase of HPLC instrument through which this study becomes possible.

Conflict of interest

None

Source of support

Nil

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