NOVEL RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF TAMSULOSIN HCl AND DUTASTERIDE IN TABLETS BY RATIO'S METHOD

Kudupudi Chandrasekhar* and A. Manikandan

Department of Science & Humanities, Bharath Institute of Higher Education and Research,
Selayur, Chennai, Tamilnadu-600073, India

✉Corresponding Author: kudupudichandra@gmail.com

ABSTRACT

A novel simple selective, precise and accurate RP-HPLC method were developed for the simultaneous estimation by ratio's method of Tamsulosin HCl and Dutasteride in the combined formulation. The drugs were estimated for the ratio's of the peak areas for Tamsulosin HCl, Dutasteride and the internal standard. The internal standard selected was propylparaben. The drugs and internal standard were separated on Kromasil C18 (250x4.6, 5 μ) with a reverse phase isocratic elution. A mixed phosphate buffer 35 volumes and acetonitrile 65 volumes were used as a mobile phase. the flow rate was 1.0 mL/min. 225 nm was the detection wavelength. The retention times were 2.804 min for Tamsulosin HCl, 4.371 min for internal standard(propylparaben) and 12.914 min for Dutasteride. The linearity ranges for Tamsulosin HCl and Dutasteride were 20.00 to 60.00 and 25 to 75 mcg/ml respectively with a correlation coefficient of 0.999 for both. The proposed ratio's method validated statistically concerning system suitability, specificity, linearity, precision, accuracy, range, robustness and ruggedness. The method was accurate, linear, precise, specific, selective and rapid suitable for the quantitative estimation of Tamsulosin HCl and Dutasteride by ratio's method.

Keywords: Ratio's Method, Tamsulosin, Dutasteride, Internal Standard, Tablets, RP-HPLC.

RASĀYAN J. Chem., Vol. 14, No.2, 2021

INTRODUCTION

Tamsulosin HCl chemically Benzenesulfonamide, 5-[2-[[2-(2-ethoxyphenoxy)ethyl] amino]propyl]-2-methoxy-, monohydrochloride, Dutasteride chemically (5 α ,17 β)-N-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-azaan-drost-1-ene-17-carboxamide. Tamsulosin HCl and Dutasteride are official in Indian, the United States and European Pharmacopoeia. But the combination of Tamsulosin HCl and Dutasteride is not official in any one of the pharmacopoeia. Tamsulosin HCl and Dutasteride combined formulation is used to treat the symptoms of an enlarged prostate gland in men, which is also called benign prostatic hyperplasia (BPH). The tablet combination dosage form in the ratio of 0.4:0.5 mg Tamsulosin HCl and Dutasteride respectively. The average weight of the tablet is about 202mg. Many formulations are available with many trade names. Many methods have been reported for this combination by HPLC⁴⁻¹², from literature reveals no internal standard method was reported to estimate the combination of Tamsulosin HCl and Dutasteride. So the proposed ratio's method simultaneously estimates the ratio of Tamsulosin HCl peak area to the internal standard (propyl paraben) peak area and the ratio of Dutasteride peak area to the internal standard (propyl paraben) peak area in a tablet formulation to correctly estimates the loss of analyte during the sample preparation.

EXPERIMENTAL

Instrumentation

The separation was carried out on Shimadzu HPLC system with quaternary gradient and UV and 20MP PDA detectors, LC solutions software and Kromasil C18 (250mmx4.6mm, 5 μ m) column.

Chemicals and Reagents

The working standards of Tamsulosin HCl and Dutasteride were provided as gift samples from Bio-Leo Analytical Labs., Hyderabad. The marketed formulation was purchased from the local market. Propyl

Rasayan J. Chem., 14(2), 665-671(2021)

<http://dx.doi.org/10.31788/RJC.2021.1425740>

This work is licensed under a CC BY 4.0 license.

paraben from sigma. Potassium dihydrogen orthophosphate, DiPotassium dihydrogen orthophosphate Water and Acetonitrile of HPLC grade from Rankem.

HPLC Conditions

The mobile phase consisting of mixed phosphate buffer and acetonitrile in the ratio 35:65 was filtered through 0.45µm PVDF membrane filter before use, degassed and pumped through the column at a flow rate of 1.0 mL/min. The column was maintained at 40°C temperature. The elution was monitored at 225nm and the run time was 20 min. The injection volume was 10µL. the column was equilibrated for about 30 min before injection of the drug solutions with the mobile phase.

Preparation of Mixed Phosphate Buffer

Dissolve 0.525 g of DiPotassium hydrogen orthophosphate and 2.67 g of Potassium dihydrogen orthophosphate in 1000 ml of water.

Preparation of Diluent

Acetonitrile and water in the ratio 3:7.

Preparation of Internal Standard Stock Solution

Accurately transferred 100mg of propyl paraben into 100ml volumetric flask dissolve and diluted to volume with diluent.

Preparation of Standard Stock Solutions

Tamsulosin HCl

Weighed Accurately 4.00 mg transferred into 10mL of volumetric flask dissolve and diluted to volume with diluent and sonicate for 10 min.

Dutasteride

Weighed Accurately 5.00 mg transferred into 10mL of volumetric flask dissolve and diluted to volume with diluent and sonicate for 10 min.

Preparation of Standard Solution

Accurately transferred 1ml each of Tamsulosin HCl, Dutasteride and internal standard stock solutions into 10ml volumetric flask dissolve and diluted to volume with diluent.

Preparation of Sample Solution

Accurately transferred sample powder equivalent to 0.8mg of Tamsulosin HCl and 1.0mg of Dutasteride into a screw-capped centrifuge tube and 10ml of water added. Heated at 40°C for 10 minutes and shake for 20minutes. To the solution, 8ml diluent was added and shake well. 2ml of internal standard stock solution added and shake well, centrifuged at 3000 rpm for 10 minutes and used the supernatant passed through 0.45µm PVDF membrane filter.

RESULTS AND DISCUSSION

System Suitability Studies

System suitability testing is an integral part of the analytical procedure, the parameters like resolution, tailing factor, theoretical plates and area %RSD are determined for a standard solution. (Fig.-1) The results are tabulated in Table-1.

Table-1: System Suitability Results

Parameters	Tamsulosin HCl	Internal Standard	Dutasteride	Limit
Retention time	2.804	4.371	12.914	--
Resolution (R)	-	11.306	29.758	R > 2
Tailing factor (T)	1.31	1.16	0.98	T < 2
Theoretical plates (N)	7365	14083	14544	N > 2000
Area %RSD	0.07	0.08	0.07	<2.0 for n≥5

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present and to provide an exact content or potency of the analyte in the sample. The test solution monitored on PDA (Fig.-2).

Fig.-1: System Suitability Chromatogram

Fig.-2: Test Chromatogram

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The method accuracy was determined based on recovery experiments. The recovery studies were carried out at three concentration levels with triplicate preparations. The percentage recovery mean recovery and standard deviation of the percentage recovery were calculated. (Table-2 and 3).

Table-2: Tamsulosin HCl Accuracy Results

Spiked Level	Sample Amount Added (mcg/ml)	Sample Amount Recovered (mcg/ml)	% Recovery	Mean % Recovery	%RSD
50%	19.9010	20.2731	101.87	100.93	1.0
50%	20.0990	20.3121	101.06		
50%	20.0792	20.0511	99.86		
100%	40.3960	40.4082	100.03	100.17	0.19
100%	40.4950	40.5274	100.08		
100%	40.1980	40.3548	100.39		

150%	60.4950	60.1684	99.46	100.35	1.62
150%	60.3960	60.0095	99.36		
150%	58.7129	60.0163	102.22		

Table-3: Dutasteride Accuracy Results

Spiked Level	Sample Amount Added (mcg/ml)	Sample Amount Recovered (mcg/ml)	% Recovery	Mean % Recovery	%RSD
50%	24.8762	24.7842	99.63	98.95	0.78
50%	25.1238	24.6489	98.11		
50%	25.0990	24.8756	99.11		
100%	50.4950	50.1012	99.22	100.12	0.91
100%	50.6188	50.6694	100.1		
100%	50.2475	50.7701	101.04		
150%	75.6188	76.6850	101.41	100.16	1.87
150%	75.4950	76.2953	101.06		
150%	73.3911	71.9233	98.00		

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The % assay calculated for six different preparations of the sample (Fig.-3 and Table-4).

Fig.-3: Overlay Precision Chromatogram

Table-4: Precision Results

S. No.	% Assay (Tamsulosin HCl)	% Assay (Dutasteride)
Preparation-1	100.03	99.22
Preparation-2	100.08	100.10
Preparation-3	100.39	101.04
Preparation-4	101.34	99.97
Preparation-5	101.30	99.84
Preparation-6	101.39	100.28
Average Assay:	100.75	100.08
Std. Deviation	0.66	0.59
%RSD	0.65	0.59

Linearity Range

The analytical procedure linearity is its ability (within a given range) to obtain test results. The results are directly proportional to the concentration (amount) of an analyte in the sample. The interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) has been demonstrated that the range of analytical procedure which has a suitable level of precision, accuracy and linearity.

Linearity was determined by plotting ratio's of the peak areas for Tamsulosin HCl, Dutasteride and the internal standard against the concentration of solutions. The linearity of Tamsulosin HCl and Dutasteride determined in the concentration ranges of 40.00-60.00 µg/mL and 25-75 µg/mL respectively. The Tamsulosin HCl regression equation is $y = 0.027x$ with a coefficient of correlation (R^2) of 0.999. The Dutasteride regression equation is $y = 0.024x - 0.010$ with a coefficient of correlation (R^2) of 0.999 and shown in Figs.-4 and 5 and the overlay linearity chromatograms shown in Fig.-6. The range of the analytical method determined for Tamsulosin HCl and Dutasteride (Table-5).

Table-5: Range Results

Parameter	Unit	Tamsulosin HCl	Dutasteride
Range	mcg/mL	20.00-60.00	25.00-75.00
Linearity	Correlation coefficient (r^2)	0.999	0.999
Accuracy at 50%	% recovery	100.93	98.95
Accuracy at 150%	% recovery	100.35	100.16
Precision at 50%	% RSD	1.00	0.78
Precision at 150%	% RSD	1.62	1.87

Fig.-4: Tamsulosin HCl Calibration Curve

Robustness and Ruggedness

The analytical procedure robustness is small, but deliberate variations in method parameters measurement. The capacity of the method to remain unaffected and indicates its reliability when compared to normal usage.

Robustness was determined a change in the flow rate ± 0.1 ml/minute, column oven $\pm 5^\circ\text{C}$, organic phase in mobile phase $\pm 5\%$ and different batch of the column with the different analyst.

Fig.-5: Dutasteride Calibration Curve

Fig.-6: Overlay Linearity Chromatogram

System suitability results were given in Table-1 and system suitability parameters are retention time, resolution, tailing, resolution, and plate count were within the acceptable limits. So, the system is suitable for the analysis. The test chromatogram extracted on PDA detector the peak Tamsulosin HCl and

Dutasteride purities found 1.0 respectively indicates no interference, so the method is specific. Tamsulosin HCl recovery was found 100.17 to 100.93% and %RSD was 0.19 to 1.62, Dutasteride recovery was found 98.95 to 100.16% and %RSD was 0.78 to 1.87 indicates the method is accurate 50 to 150% of the target concentration. Six different %assay preparation values of the same homogeneous samples found 100.03 to 101.39% for Tamsulosin HCl and 99.22 to 101.04% for Dutasteride indicates the method is precise. Tamsulosin HCl Linear correlation was found to be $y = 0.027x$, the correlation coefficient was 0.999 and Dutasteride was $y = 0.024x - 0.010$ and correlation coefficient was 0.999 indicates the method is linear across the target concentration. The method unaffected due to deliberate changes in flow rate, column oven temperature and different batch columns with different analysts proves the method is robust and rugged. The LOD and LOQ values were found to be 0.13 and 0.4mcg/mL for Tamsulosin HCl and 0.17 and 0.5 mcg/mL for Dutasteride respectively. The proposed range 50 to 150% of the target concentration i.e., 20.00 to 60.00 mcg/mL for Tamsulosin HCl and 25.00 to 75.00 mcg/mL for Dutasteride found linear, accurate and precise (Table-5).

CONCLUSION

A new, specific, selective, linear, accurate and precise isocratic RP-HPLC ratio's method was developed for the estimation of Tamsulosin HCl and Dutasteride in their pharmaceutical tablet formulation. The proposed method was successfully separated Tamsulosin HCl, Dutasteride and internal standard. The Proposed method is specific, selective, and stability-indicating power. Hence the developed method could be adapted to regular quality control analysis and stability analysis.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Science & Humanities, M/s Bharat Institute of Higher education and research, Chennai India for encouragement.

REFERENCES

1. Indian Pharmacopoeia, Indian Pharmacopoeia Commission, Ghaziabad, p.1633,2824(2014).
2. United States of Pharmacopoeia and National Formulary, The United States Pharmacopeial Convention, Rockville, p.1535,4169(2019).
3. European Pharmacopoeia, European Directorate for the Quality of Medicines & HealthCare, Council of Europe, France, p.2118,3364(2014).
4. G. Sravan Kumar Reddy, S. Ashutosh Kumar, Manidipa Debnath, Viriyala Raj Kumar, *International Journal of Pharmacy and Pharmaceutical Sciences*, **6**, 77 (2014).
5. B. Mohammed Ishaq, K. Vanitha Prakash and G. Krishna Mohan, *Der Pharma Chemica*, **6**, 103 (2014).
6. Dendukuri V. L. N. Mrudula, G. Sai Prasad, P. V. Rao, S. Manohar Babu, *International Journal of Research in Pharmaceutical and Nano Sciences*, **3**, 242 (2014).
7. L. Shivakumar Reddy, S.L.N. Prasad Reddy And G.Srinivas Reddy, *Oriental Journal of Chemistry*, **29**, 1665 (2013), DOI:10.13005/ojc/290456
8. Y. Sowmya, P. Aleti, R. K. Venisetty, *International Journal of Pharmacy and Biological Sciences*, **3**, 301 (2013).
9. P. Giriraj, T. Siva K. Kumar, *International Journal of Drug Development and Research*, **6**, 36 (2014).
10. Jahnavi Bandla, Gorja Ashok, *World Journal of Pharmaceutical Research*, **3**, 4113 (2014).
11. V. Mohan Goud, A. Srinivasa Rao, S. V. Phanindra, S. Pragati Ranjan, K. Praveen Reddy, *Asian Journal of Pharmaceutical and Clinical Research*, **2**, 570 (2014).
12. P. Nagaraju, B. Durga Prasad, G. Indira Priyadarshini, *Advance Pharmaceutical Journal*, **2**, 134 (2017).
13. ICH Q2 (R1), Validation of Analytical Procedure: Text and Methodology, November 2005.

[RJC-5740/2020]