IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
February 2020 Vol.:14, Issue:4

Method Development and Validation of Dutasteride and Tamsulosin by RP-HPLC

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Utla Srilatha*, Anil Kumar V[1]

**, 1 Department of Pharmaceutical Analysis, Sahasra Institute of Pharmaceutical Sciences, Warangal, Telangana-506007*

Submission: 21 January 2020
Accepted: 29 January 2020
Published: 29 February 2020

HUMAN JOURNALS
www.ijsrm.humanjournals.com

Keywords: Tamsulosine, Dutasteride, Wavelength, Calibration and Retention Time

ABSTRACT

A newer, sensitive, simple, accurate and low cost RP-HPLC method was developed for the estimation of Tamsulosine and Dutasteride. Initially, various mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with buffer: acetonitrile (43:57) with 1 ml/min flow rate is quite robust. The optimum wavelength for detection was 254 nm at which better detector response for drug was obtained. The average retention time for Tamsulosine and Dutasteride were found to be 2.2 and 5.8 min. System suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 60-100 µg/ml**.** The low values of %R.S.D indicate the method is precise and accurate. The mean recoveries were found in the range of 99.8%-101.4% and 98.6%-100.0%. Sample to sample precision and accuracy were evaluated using three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days, over a period of three days.

INTRODUCTION

Reversed Phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With these stationary phases, Retention Time (RT) is longer for molecules which are more non-polar, while polar molecules elute more readily. An investigator can increase RT by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger relative to the now more hydrophilic mobile phase. Similarly, an investigator can decrease retention time by adding more organic solvent to the eluent. RPC is so commonly used that it is often incorrectly referred to as "HPLC" without further specification. The pharmaceutical industry regularly employs RPC to qualify drugs before their release.

Method development and optimization in liquid chromatography is still an attractive field for theoreticians and attracts also a lot of interest from practical analysts. Among all, the liquid chromatographic methods, the reversed phase systems based on modified silica offers the highest probability of successful results. However, a large number of (system) variables (parameters) affect the selectivity and the resolution. Alternate analytical methods are developed for the drug product to reduce the cost and time. When alternative analytical methods are intended to replace the existing procedure, analyst should collect the literature for all types of information related to analyte and define the separation goal. Then estimate the best separation condition from trial runs. After optimizing the separation condition, validate the method for release to routine laboratory.

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, strength and quality, for the quantification of the drug substances and drug products. Method validation has received considerable attention in the literature and from industrial committees and regulatory agencies. Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and drug products. The real goal of validation process is to challenge the

method and determine the limits of allowed variability for the conditions needed to run the method.

MATERIALS AND METHODS

INSTRUMENTS REQUIRED:

Table No. 1: List of Instruments Used

Sr. No.	Instruments/Equipments/Apparatus
1	WATERS with autochrome 3000 Software with separation module 2695
2	ELICO SL-159 UV – Vis spectrophotometer
3	Electronic Balance (SHIMADZU AUX 200)
4	Ultra Sonicator (Wensar wuc-2L)
5	Column symmetry c8(4.6×150 mm, 3.5 µm, Make: X Terra)
6	pH Analyzer (ELICO)

CHEMICALS, REAGENTS AND STANDARDS REQUIRED:

Table No. 2: List of Chemicals, Reagents and Standards

Sr. No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1	Doubled distilled water	----	----	Sd fine-Chem ltd; Mumbai
2	Methanol	99.9%	A.R.	Loba Chem; Mumbai
3	Ortho phosphoric acid	99.0%	G.R.	Sd fine-Chem ltd; Mumbai
4	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai
5	Potassium dihydrogen orthophosphate	99.9%	G.R.	Sd fine-Chem ltd; Mumbai
6	Sodium hydroxide	99.9%	G.R.	Sd fine-Chem ltd; Mumbai

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Equipment : HPLC equipped with Auto Sampler and PDA detector

Column : Symmetry C8 (4.6 x 150mm, 3.5µm, Make: XTerra)

Flow rate : 1 mL per min

Wavelength : 254 nm

Injection volume : 20 µl

Column oven : Ambient

Run time : 8min

Preparation of Phosphate buffer:

Weigh 7.0 grams of KH_2PO_4 into a 1000ml beaker, dissolve and diluted to 1000ml with HPLC water. Adjust the pH to 4.5 with Orthophosphoric acid. Filter through 0.45µm nylon membrane filter and degas.

Preparation of mobile phase:

The mobile phase is prepared by mixing a mixture of above buffer 43ml of pH 4.5 phosphate buffer and 57ml of Methanol (HPLC grade) in 100ml of volumetric flask.

Preparation of standard solution:

Accurately weigh and transfer 25 mg of Tamsulosin & 25mg of Dutasteride working standard into a 100 mL volumetric flask add about 70 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 2 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Preparation of Sample Solution:

Weigh 4 tablets of Tamsulosin and Dutasteride and calculate the average weight, weigh accurately and transfer the sample equivalent to 25 mg of Tamsulosin and 25mg of Dutasteride into a 100 mL volumetric flask. Add about 70 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

RESULTS

OPTIMIZED METHOD (Buffer 6.8: Acetonitrile (43:57)):

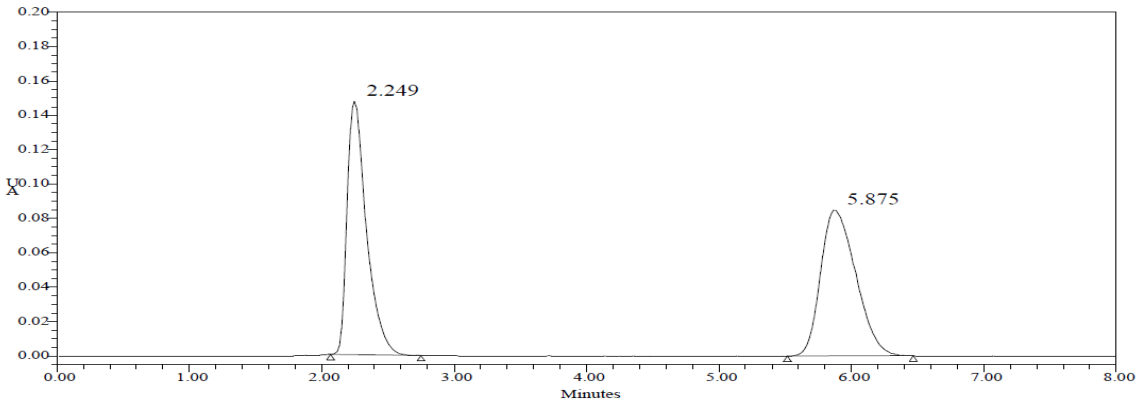


Figure No. 1: Chromatogram of Tamsulosin and Dutasteride in optimized method

Sr. No.	Name	Retention Time (min)	Area (µv*sec)	Height(µv)
1	Tamsulosine	2.249	1321978	128745
2	Dutasteride	5.875	1359867	87947

Linearity:

Linearity of Tamsulosine:

Table No. 3: Linearity of Tamsulosin

Sr. No.	Linearity Level	Concentration	Area
1	I	60ppm	1189032
2	II	70ppm	1497165
3	III	80ppm	1851004
4	IV	90ppm	2186380
5	V	100ppm	2548658
Correlation Coefficient			0.999

Linearity graph of Tamsulosin:

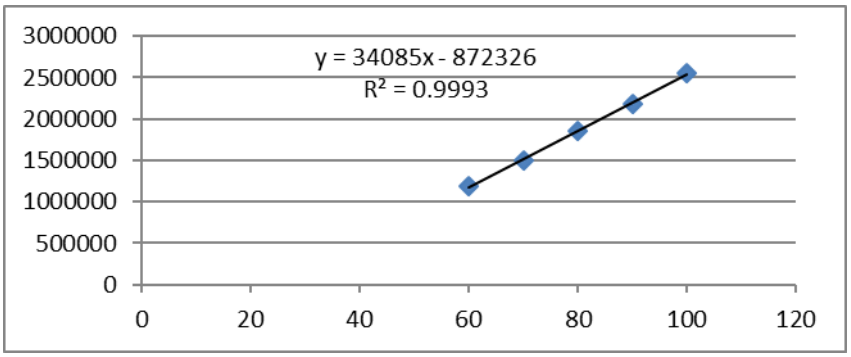


Figure No. 2: Linearity of Tamsulosin

Linearity of Dutasteride:

Table No. 4: Linearity of Dutasteride

Sr. No.	Linearity Level	Concentration	Area
1	I	60ppm	1219297
2	II	70ppm	1531332
3	III	80ppm	1856868
4	IV	90ppm	2163060
5	V	100ppm	2530502
Correlation Coefficient			0.999

Linearity graph of Dutasteride:

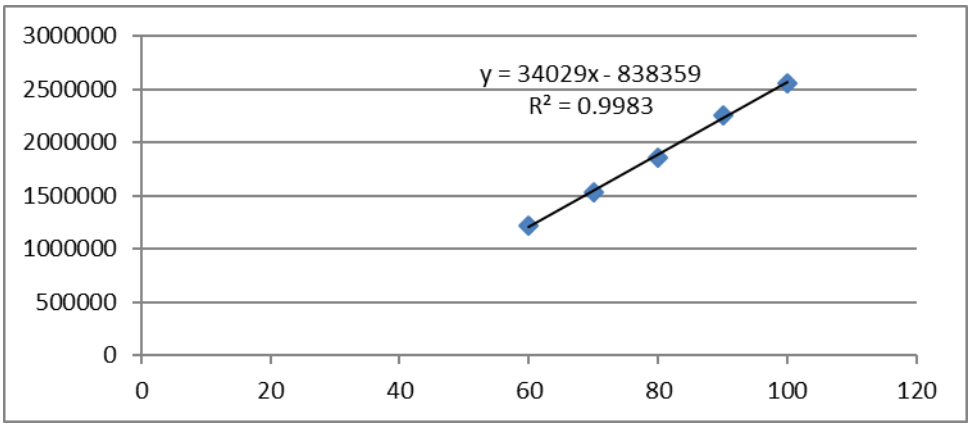


Figure No. 3: Calibration curve for linearity of Dutasteride

Assay:

Tamsulosin and Dutasteride:

Table No. 5: Assay of Tamsulosin and Dutasteride

Sr. No.	Tamsulosin		Dutasteride	
01	Spl. Area	1525384	Spl. Area	1503.654
02	Std. Area	1532594	Std. Area	1457.193
03	Std. Wt	10mg	Std. Wt	10mg
04	Spl. Wt	16.98mg	Spl. Wt	16.98mg
05	LC	500mg	LC	85mg
06	Avg. Wt	993.7mg	Avg. Wt	993.7mg
07	Std. Purity	99.8	Std. Purity	99.6
08	Assay %	98.8	Assay %	98.6

ACCURACY

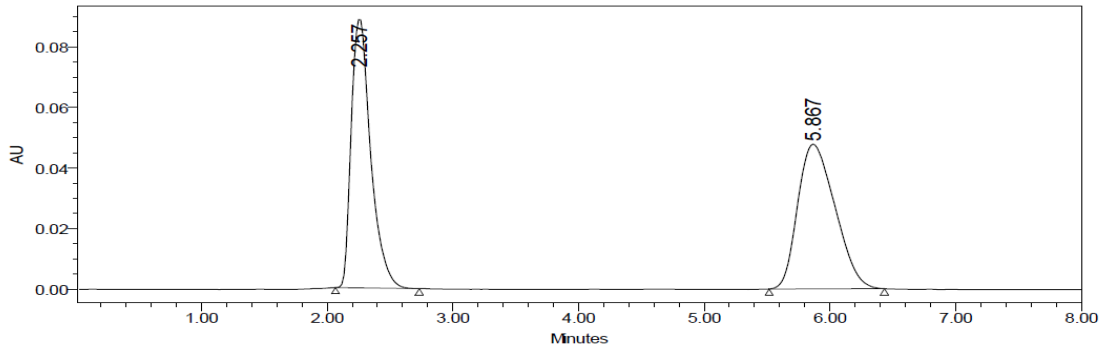
Table No. 6: Accuracy of Tamsulosin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	984243	5.0	5.07	101.4%	100.56%
100%	1567396	10.0	9.98	99.8%	
150%	2497228	15.0	15.08	100.5%	

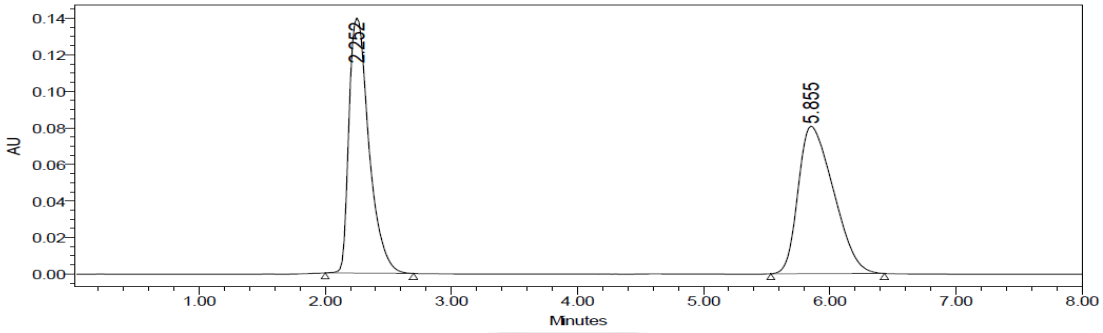
Table No. 7: Accuracy of Dutasteride

%Concentration	Area	Amount Added(mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	958196	5.0	4.97	98.6%	99.4%
100%	1532695	10.0	9.96	99.6%	
150%	2425792	15.0	15.01	100.%	

Accuracy (50%):



Accuracy (100%):



Accuracy (150%):

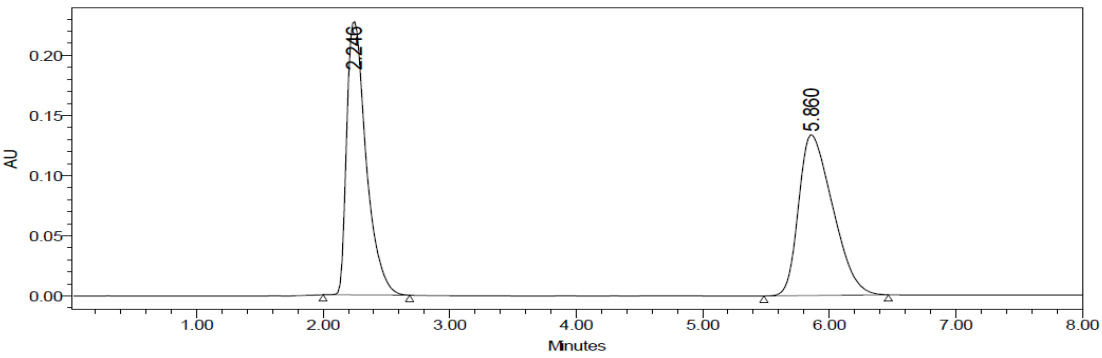


Figure No. 4: Chromatograms of Accuracy

DISCUSSION

An effort has been made to identify a simple, precise, specific and accurate method for the estimation of Dutasteride and Tamsulosin in formulations by using RP-HPLC method.

As a part of trial and error method to get the optimized chromatographic conditions, various proportions of buffers and mobile phase composition was tried. During trial and error methods the retention time found was low and the peak was asymmetric. For some of the composition of buffers and mobile phase, the retention time and resolution was too long and the peak was symmetric. For optimized method the retention time and shape was good, hence t5he optimized method was finalized for the estimation of Dutasteride and Tamsulosin.

A simple reverse phase HPLC method was developed for the determination of Dutasteride and Tamsulosin. Symmetry C8 (4.6 x 150mm, 3.5µm, Make: XTerra) in an isocratic mode with mobile phase Phosphate buffer (ph-4.5): Acetonitrile (43:57) was used. The flow rate was 1 ml/ min and effluent was monitored at 254 nm. The retention time for Tamsulosin and Dutasteride is 2.2min and 5.8min respectively.

As the results are within the acceptance limits for linearity, the proposed method is found to be linear at concentration of 5-25 µg/ml for Tamsulosin. As the results are within the acceptance limits, the proposed method is found be linear at concentration of 5-25 µg/ml for Dutasteride.

As the results are within the acceptance limits of 98-102% for its accuracy the proposed method is accurate.

CONCLUSION

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost RP-HPLC method. It is successfully applied for the determination of Tamsulosin and Dutasteride in pharmaceutical preparations without the interferences of other constituent in the formulations.

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was based on peak parameters (height,

tailing, theoretical plates, capacity factor), run time etc. The system with Buffer: acetonitrile (43:57) with 1 ml/min flow rate is quite robust.

The optimum wavelength for detection was 254 nm at which better detector response for drug was obtained. The average retention time for Tamsulosin and Dutasteride were found to be 2.2 and 5.8min. System suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 60 – 100 μg/ml**.** The low values of % R.S.D. indicate the method is precise and accurate. The mean recoveries were found in the range of 99.8% – 101.4% and 98.6%-100%.

Sample to sample precision and accuracy were evaluated using, three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days, over a period of three days. These results show the accuracy and reproducibility of the assay.

ACKNOWLEDGEMENTS

I humbly present this work to the eternal almighty. Indeed my final work is done with the help of primitive persons at heart. So it is my bounded duty to promulgate them individually.

I wish to extend my sincere thanks to my beloved guide Dr. Anil Kumar V, Associate Professor, Department of Pharmaceutical Analysis, Sahasra Institute of Pharmaceutical Sciences, Warangal for extending his valuable guidance and encouragement throughout the work.

ABBREVIATIONS

HPLC	High performance Liquid Chromatography
%	Percent
PDA	Photodiode Array
ICH	International Conference for Harmonization
GR	General reagent
C18	Octadecyl
UV	Ultraviolet
ml	Milliliter
Min	Minute
MeOH	Methanol
µl	Micro Liter
µ	Micron
µg	Micro gram
ppm	Parts per million
nm	Nanometer
RSD	Relative Standard Deviation
Fig	Figure

REFERENCES

1. Gurdeep R Chatwal and Sham K Anand, "Instrumental Methods of Chemical Analysis 5th Edition, Himalaya Publishing House, Page No. 1.2-1.5.
2. Ewing, G.W, "Instrumental Methods of Chemical Analysis", 2nd Edition, 1960, MC Graw Hill Book Company, Page No. 1-3.
3. James W Munson, "Modern Methods of Pharmaceutical Analysis, 2001, Medical Book Distributors, Mumbai. Page No. 17-54.
4. Sethi P.D. HPLC 'High Performance Liquid Chromatography and Quantitative analysis Pharmaceutical Formulations", 1st Edition, CBS Publishers and Distributors; 2001. Page No. 3-5.
5. Erwing, G. W., "Instrumental Methods of Chemical Analysis,' 2nd Edn., 1960 McGraw Hill Book Company, Page No. 3-5.
6. Jenkins GL., Knevel, A.M and Digangi, F. E., "Quantitative Pharmaceutical Chemistry". 7th Edn. McGraw Hill Book Co., New York, Edition 1977, Page No. 297-299.
7. Beckett A. H and Stenlake J. B., "Practical Pharmaceutical Chemistry" 4th Edition. Part II, CBS Publisher and Distributor, New Delhi, 1997, Page No. 277-278.
8. Bently and Drivers, "Textbook of Pharmaceutical Chemistry," 8th Edition, 1985, O'Brein, Oxford University Press, Page No. 1-3.
9. Napoleon A.A. "Pharmaceutical Titrimetric Analysis theory and practical," Kalaimani Publishers and Distributors, Kanchipuram, Page No. 1.1-1.4.