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SIMULTANEOUS ESTIMATION OF DUTASTERIDE AND TAMSULOSIN HCL PHARMACEUTICAL FORMULATIONS BY RP-HPLC

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ABSTRACT

The Purpose of this work to develop an accurate, simple, sensitive and precise RP-HPLC method was developed for the determination of Dutasteride and Tamsulosin hydrochloride in tablet dosage form. The RP-HPLC separation was achieved on BDS Hypersil C18 column (250 mm, id 4.6 mm, 5 µm) using mobile phase CH3COONH4: Methanol (550:450)v/v) at a flow rate of 0.8 ml/min at an 30 ^{0}C temperature. Quantification was achieved with photodiode array detection at 254 nm over the concentration range 80µg/ml. The propose method was validated for its linearity. Statistically, accuracy, precision and robustness. This method can be employed for routine quality control analysis of Dutasteride and Tamsulosin Hydrochloride in tablet dosage form, and also applied successfully for the determination of dutasteride and Tamsulosin Hydrochloride in combination of pharmaceutical dosage form.

KEYWORDS

Tamsulosin Hcl, Dutasteride and RP-HPLC.

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INTRODUCTION

Dutasteride inhibits the formation of 5 alpha-dihydrotestosterone (DHT) from testosterone, which is the androgen primarily responsible for the initial development and subsequent enlargement of the prostate gland. DHT formed from testosterone by the action of enzyme 5 alpha-reductase, which exists in 2 isoforms, type 1 and type 2. Dutasteride inhibits both type 1 and type 2 5 alpha-reductase isoenzymes competitively, with which it forms a stable enzyme complex. Dissociation from this complex has been evaluated under *in vitro* and in

vivo conditions and is extremely slow. Dutasteride will not bind to the human androgen receptor.

Tamsulosin is a selective antagonist of alpha-1A and alpha-1B-adrenoceptors present in the prostate, prostatic urethra, bladder neck and also prostatic capsule. Three alpha1-adrenoceptor subtypes have been identified and they arealpha-1A, alpha-1B and alpha-1D. Approximately 70% of the alpha1-receptors in human prostate are alpha-1A subtype. Blockage of these receptors causes relaxation of smooth muscles in the bladder neck and prostate, and thus decreases urinary outflow resistance in men.

The technique of High Performance Liquid Chromatography is so called because of its improvement performance when compared to classical column chromatography. It is also called as High pressure liquid chromatography since high pressure is used when compared to classical column chromatography. The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process, if diffusion is minimized, a faster and effective separation can be achieved.

In the present work, attempts were made to develop analytical method of simultaneous estimation of Dutasteride and Tamsulosin Hcl pharmaceutical formulations by RP - HPLC method.

MATERIAL AND METHODS[3-5]

Instruments

WATERS HPLC, Model: Agilent 2695, Photo diode array detector (PDA), with an automated sample injector. The output signal was monitored and integrated using Empower 2 software. ELIPSE C8 (150mm* 4.6, 5 µm, Make: Waters) column was used for separations. List of instruments are listed in Table No.1 and chemicals are listed in Table No.2.

OPTIMIZED METHOD

Chromatographic parameters

Mobile Phase: CH3COONH4: Methanol (550:450)
Column: BDS Hypersil 250X4.6mm, C18, 5um
Flow Rate: 0.8ml/min
Temperature: 30^0c
Volume: 10ul
Detector: PDA

Procedure

Inject 10µL of standard, sample into chromatographic system and measure the areas for the Dutasteride and Tamsulosin Hcl peaks and calculate the % assay by using the formula

Preparation of Mobile Phase[6,7]

Transfers 1000ml of HPLC water into 1000ml of beaker add Ammonium Acetate.

Transfer the above solution 700mlof CH3COONH4, 300ml of Methanol is used as mobile phase. They are mixed and sonicated for 20min.

PREPARATION OF THE DUTASTERIDE AND TAMSULOSIN HCLSTANDARD AND SAMPLE SOLUTION

Preparation of Standard Solution

Accurately weigh and transfer10mg of Dutasteride and 8.13mg TamsulosinHclinto50ml of volumetric flask and add 10ml of Methanol and sonicate 10min (or) shake 5min and make with water.

Transfers the above solution into 5ml into 25ml volumetric flask dilute to volume with water.

Preparation of Sample Stock Solution

Commercially available 20 tablets were weighed and powdered the powdered equivalent to the 2650.5mg of Dutasteride and Tamsulosin Hcl of active ingredients were transfer into a 25ml of volumetric flask and add 10ml of Methanol and sonicate 20min (or) shake 10min and makeup with water.

Transfers above solution 5ml into 25ml of the volumetric flask dilute the volume with Water. And the solution was filtered through 0.45µm filter before injecting into HPLC system.

METHOD VALIDATION[8-10]

System Suitability

Tailing factor for the peaks due to Dutasteride and Tamsulosin Hcl in standard solution should not be more than 2.0. Theoretical plates for the peaks Dutasteride and Tamsulosin Hcl in standard solution should not be less than 2000.

Specificity

Solution of standard, sample, blank and placebo were prepared as per test procedure and injected into the HPLC system.

Acceptance criteria

Chromatogram of standard and sample should be identical with near Retention time.

Blank interference

A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure.

Acceptance criteria

Chromatogram of blank should not show any peak at the retention time of analyte peak. There is no interference due to blank at the retention time of analyte. Hence the method is specific.

LINEARITY

Prepare a series of standard solutions and inject into HPLC system. Plot the graph of standard versus the actual concentration in µg/ml and determine the coefficient of correlation and basis for 100% response.

Acceptance criteria

Linearity regression coefficient of average peak area response of replicate injections plotted against respective concentration should not be less than 0.999. The % y-intercept as obtained from the linearity data (without extrapolation through origin 0, 0) should be within ±2.0.

Statistical Evaluation

A graph between the concentration and the average area was plotted. Points for linearity were observed. Using the method of least squares, a line of best fit was taken and the correlation Coefficient, slope and, y-intercept were calculated.

PRECISION

Preparation of sample

- Transfer the 2650.5mg of sample into a 50ml of volume at flask and add 10ml of water and 10ml of Methanol and sonicate 20min and makeup with water. Transfer the above solution into 5ml into 25ml volume metric flask dilute to the volume with water.
- The method precision parameters were evaluated from sample chromatograms obtained, by calculating the % RSD of peek areas from 6 replicate injections.

Acceptance criteria

The injection reproducibility requirements are met if the %RSD for peak areas is not more than 2.0 and for retention time is are not more than 2.0.

RECOVERY/ACCURACY

Recovery study can be performed in the concentration range of 80% to 120% of the target concentration of the test. Minimum 3 concentrations are recommended.

Acceptance criteria

The average percentage recovery was between 98-102% and Relative standard deviation of these recovery concentrations was less than 2%.

Limit of Detection

The sensitivity of measurement of Dutasteride and Tamsulosin Hcl by use of proposed method was estimated in terms of the limit of detection (LOD). The LOD was calculated by the use of signal to noise ratio. In order to estimate the LOD value, the blank sample was injected six times and peak area of this blank was calculated as noice level. The LOD was calculated as three times the noise level.

$$LOD = 3.3\ \sigma / S$$

Where,

σ = standard deviation of intercepts of calibration curves

S = mean of slopes of the calibration curves

The slope S may be estimated from the calibration curve of the analyte.

Limit of Quantitation

The sensitivity of measurement of Dutasteride and Tamsulosin Hcl by the use of proposed method was estimated in terms of limit of quantitation (LOQ). The LOQ was calculated by the use of signal to noise ratio. In order to estimate the LOQ value, the blank sample was injected six times and the peak area of this blank was calculated at noise level. The LOQ was calculated as ten times the noise value gave the LOQ.

$$LOQ = 10\ \sigma / S$$

Where,

σ = standard deviation of intercepts of calibration curves

S = mean of slopes of the calibration curves

The slope S may be estimated from the calibration curve of the analyte.

ROBUSTNESS

Effect of variation in flow rate

Prepare the system suitability solution as per the test method and inject into the HPLC system with ±0.2 ml of the method flow. Evaluate the system suitability values as required by the test method for both flow rates.

Actual flow rate was 1.0 ml/min and it was changed to 0.8ml/min and 1.2ml/min and inject into HPLC and system suitability was checked.

Effect of variation in wavelength

Prepare the system suitability solution as per the test method and injected into the HPLC with ±2nm variation in wavelength. Evaluate the system suitability values as required by the test method for both wavelengths.

Observation

Peaks are well separated all the parameters are within the limits. For quantitative analytical purpose wavelength was set at 254nm, which provided better reproducibility.

Table No.1: List of Equipment's

S.No	Equipment's	Model	Company
1	Electronic Balance	ER200A	ASCOSET
2	Ultra-Sonicator	SE60US	ENERTECH
3	Heating Mantle	BTI	BIO TECHNICS INDIA
4	Thermal oven	---------	NARANG
5	pH Meter	AD102U	ADWA
6	Filter Paper 0.45 microns	---------	MILLI PORE

Table No.2: List of chemicals and reagents used

S.No	Chemicals/standards and reagents	Grade	Make
1	Ammonium Acetate	AR	Finar
2	Methanol	HPLC	Merck
3	Water	HPLC	Loba Chemi
4	Sodium Di Hydrogen Ortho Phosphate	AR	Hetero
5	Dutasteride	NA	Hetero
6	Tamsulosin Hcl	NA	Hetero

Table No.3: Validation data for Dutasteride

S.No	Parameter	Result	Acceptance criteria
1	System suitability Theoretical plates Asymmetry Retention time %RSD	4021 1.36 3.171 0.3	Not less than 2500 Not more than2 Not more than 2%
2	Specificity a) Blank interference b) Placebo interference	Specific	Specific
3	Method precision(%RSD)	0.10	Not more than 2.0%
4	Linearity parameter Slope Intercept Correlation coefficient(r^2)	1000-3000mcg/ml 0.999	Not less than 0.999
5	Accuracy (Mean % recovery) 50% 100% 150%	100% 100% 100%	97.00 – 103.00%
6	Robustness a) Flow rate variation b) Temperature variation	All the system suitability parameters are within the limits.	

Table No.4: Validation data for Tamsulosin Hcl

S.No	Parameter	Result	Acceptance criteria
1	System suitability Theoretical plates Asymmetry Retention time %RSD	3700 1.23 4.426 0.5	Not less than 2000 Not more than 2 Not more than 2
2	Specificity c) Blank interference d) Placebo interference	Specific	Specific
3	Method precision(%RSD)	0.03	Not more than 2.0%
4	Linearity parameter Slope Intercept Correlation coefficient(r^2)	50-150 mcg/ml 0.999	Not less than 0.999
5	Accuracy (Mean % recovery) 50% 100% 150%	100 100 100	97 - 103%
6	Robustness c) Flow rate variation d) Temperature variation	All the system suitability parameters are within the limits.	

S.No	Name	Retention Time	Area	USP Resolution	USP Tailing	USP Plate Count
1		3.118	4185507		1.42	3859
2		4.296	7479902	4.73	1.28	3583

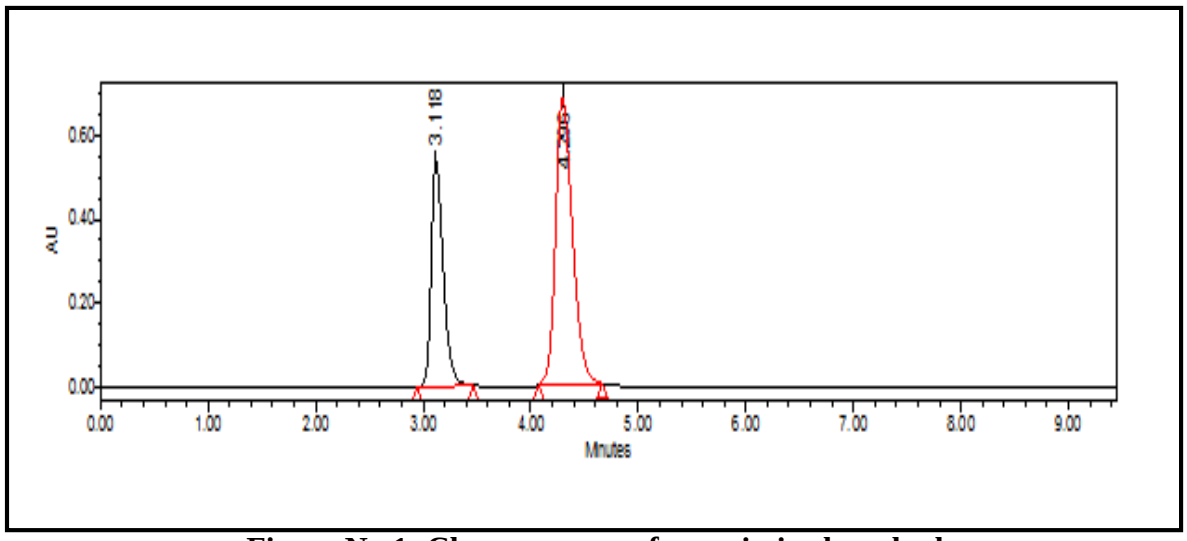

Figure No.1: Chromatogram for optimized method

CONCLUSION

From the reported literature, there were few methods established for the determination of Dutasteride and Tamsulosin Hcl individual and in combination with other drug.

It was concluded that there was reported for the simultaneous estimation of the above selected multi component dosage form, which promote to pursue the present work. The scope and objective of the present work is to develop and validate a new simple RP-HPLC method for simultaneous estimation of Dutasteride and Tamsulosin Hcl combined dosage form.

In simultaneous RP-HPLC method development, Waters HPLC with PDA detector and column used is BDS Hypersil 250X 4.6mm, C18with 5-micron particle size. Injection volume of 10 µL is injected and eluted with the mobile phase selected after optimization was 0.1M CH3COONH4 and Methanol in the ratio of 55:45 was found to be ideal. The flow rate was found to be optimized at 0.8 mL/min. Detection was carried out at 254nm. Quantitation was done by external standard method with the above mentioned optimized chromatographic condition. This system produced symmetric peak shape, good resolution and reasonable retention times of Dutasteride and Tamsulosin Hcl were found to be3.118 and4.296 minutes respectively.

The Dutasteride and Tamsulosin Hcl showed linearity in the range of 1000-3000 µg/mL and 50-150 respectively. The slope and correlation coefficient values for Dutasteride were found to be 43363 and 0.999 respectively and 13168 and 0.999 respectively for Tamsulosin Hcl which indicates excellent correlation between response factor Vs concentration of standard solutions.

Precision of the developed method was studied under system precision and method precision. The %RSD values for precision was found to be within the acceptable limit, which revealed that the developed method was precise. The developed method was found to be robust. The %RSD value for percentage recovery Dutasteride and Tamsulosin Hcl were found to be within the acceptance criteria. The results indicate satisfactory accuracy of method for simultaneous estimation of the Dutasteride and Tamsulosin Hcl. The forced degradation study showed the method was highly specific

From the above experimental data and results, the developed HPLC method is having the following advantages:

- The standard and sample preparation requires less time.
- No tedious extraction procedure was involved in the analysis of formulation.
- Run time required for recording chromatograms were less than 10 minutes.

- Suitable for the analysis of raw materials, applicable to dissolution studies and can be used for the content uniformity studies.

Hence, the chromatographic method developed for the Dutasteride and Tamsulosin Hcl said to be rapid, simple, sensitive, precise and accurate that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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