EXPERIMENTAL WORK on SIMULTAMEOUS ESTIMATION OF DUTASTERIDE AND TAMSULOSINE BY RP-HPLC METHOD IN PHARMACEUTICALDOSAGE FORM

MANUKONDU BHASKAR
Assistant Professor
Department of Pharmaceutical chemistry
VIJAYA college of Pharmacy
Hyderabad.

Abstract

Dutasteride (DTS), selective inhibitor of both, type 1 and type 2 isoforms of 5α-reductase (5-AR) enzyme that converts testosterone to 5α-dihydrotestosterone (DHT) which is responsible for enlargement of prostate, is used in treatment of benign prostatic hyperplasia, frequently occurring in men over the age of 50 years[1]. Chemically, DTS is (5α,17β)-N-{2,5 bis (trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide with an empirical formula $C_{27}H_{30}F_6N_2O_2$, representing a molecular weight of 528.5 g/mol[2]. Literature survey revealed LC-MS and HPLC methods for estimation of DTS in human plasma and pharmaceutical dosage forms[3–5]. A LC-MS-MS method is reported for the simultaneous determination of tamsulosin and dutasteride in human plasma[6]. So it was thought of interest to develop a simple and sensitive RP-HPLC method for determination of DTS in tablet.

Introduction

All the reagents used were of HPLC grade and analytical grade. Reference standard of DTS was procured from Intaas Pharmaceutical Limited, Ahmedabad with 99.98% purity. Tablets of two different batches of Veltride (0.5 mg) of Intaas Pharmaceutical Ltd. were purchased from a local pharmacy. A standard stock solution of DTS (1 mg/ml) was prepared by dissolving 25 mg of drug in 25 ml methanol. Working standard solution (100 µg/ml) was prepared from stock solution by proper dilution with methanol.

A Shimadzu HPLC (LC-2010HT-liquid chromatograph) equipped with PDA detector (SPD-M20A), phenomenox (Torrance, CA) C_{18} (250×4.6 mm i.d., 5 µm) column and LC solution software were used. The mobile phase used was methanol:water (90:10, v/v) which was filtered through nylon 0.45 µm membrane filter and degassed by ultrasonication for 15 min.

Linearity of the method was investigated by serially diluting the working standard to give a concentration range of 1-12 µg/ml and 20 µl from this solution was injected. The flow rate was maintained at 1 ml/min. Temperature of the column was kept at ambient and the effluent was monitored at 235 nm. Calibration curve was constructed by plotting concentration against peak area. The method was validated for linearity, precision, accuracy, and specificity, limit of detection and limit of quantification as per ICH guidelines[7].

Assay of tablets of DTS were performed. Thirty tablets of each batch having 0.5 mg strength were weighed and ground to a fine powder. A quantity of tablet powder equivalent to 10 mg of DTS was transferred to 10 ml volumetric flask, dissolved and diluted with methanol to obtained 1 mg/ml. The solution was sonicated for 15 min and filtered through 0.45 µm membrane filter. The solution was further diluted to obtain concentration 10 µg/ml. Peak area of the above prepared tablet solutions of DTS were measured by using above mentioned chromatographic conditions and the amount of DTS were found from regression equation.

To optimize the HPLC parameters, several mobile phase compositions were tried. Various mobile phases having different ratios of methanol, water and acetonitrile were tried. Drug was retained in mobile phase consisting of acetonitrile:water

(60:40, v/v) and methanol:water (60:40, v/v). In acetonitrile:water (90:10, v/v) tailing in the peak was observed. Good peak symmetry and satisfactory retention time was obtained with mobile phase consisting of methanol:water (90:10 v/v). Quantification was achieved with PDA detection at 235 nm based on peak area. The retention time of DTS obtained was 5.24±0.112 (fig. 1). The system suitability tests for HPLC were carried out on freshly prepared solution of DTS (10 µg/ml) and the parameters were studied.

INSTRUMENTS USED

Table - Instruments used

S.No	Instrument	Model
1	HPLC	WATERS, software: EMpower, 2695 separation module.2487 UV detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000+
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

CHEMICALS USED

Table - Chemicals used

S.No	Chemical	Brand
1	Dutasteride	Dr. Reddy's
2	Tamsulosin	Dr. Reddy's
3	KH_2PO_4	Finer chemical Ltd
4	Water and Methanol for HPLC	Lichrosolv (Merch)
5	Acetonitrile for HPLC	Molychem
6	HCl, H_2O_2, NaOH	Merck

HPLC METHOD DEVELOPMENT

Mobile phase optimization

To begin with the cell phase tried became methanol: Ammonium acetate buffer and acetonitrile: phosphate buffer with various mixtures of pH in addition to varying proportions. in the end, the mobile section was optimized to potassium dihydrogen phosphate with buffer (pH 2.5), acetonitrile in percentage 20: eighty V/V respectively.

Wave length selection

UV spectrum of 10 µg / ml DUTA and TAM in diluents was recorded via scanning inside the range of 200nm to 400nm. From the UV spectrum, the wavelength of 274 nm turned into decided on. At this wavelength DUTA and TAM standards suggests right absorbance.

Optimization of Column

The approach become carried out with diverse columns like hypersil column, lichrosorb, and inertsil ODS column. C18 column of XTerra (150mm x four.6) become found to be perfect as it gave a terrific height shape and resolution at 0.8ml/min waft.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Temperature:Ambient

Column:Symmetry C18 (4.6 x 150mm, 5□m,

Make:XTerra) or equivalent

Buffer:7.0 grams of potassium dihydrogen ortho phosphate in

1000 ml water pH adjusted with ortho phaosparic acid.

pH:2.5

Mobile phase:phosphate Buffer: acetonitrile (20: 80 v/v)Flow rate : 0.8 ml per min

Wavelength : 274 nm

Injection

volume : 20 □l Run
time : 7min.

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Phosphate buffer

As it should be weighed 7.zero grams of potassium dihydrogen ortho phosphate is taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and the quantity become adjusted to pH 2.5 with Orthophosphoric acid.

Preparation of mobile phase

Appropriately measured two hundred ml (20%) of above buffer and 800 ml of Acetonitrile HPLC (80%) have been combined and degassed in an ultrasonic water bathtub for 10 mins and then filtered via zero.45 µ clear out beneath vacuum filtration. This changed into used as diluent.

VALIDATION PARAMETERS

Method Precision

Standard Solution Preparation

Appropriately weighed amount of 50mg DUTA and 50 mg TAM have been taken to a hundred ml clean and dry volumetric flask. This was then diluted with 70 ml of diluent and turned into sonicated. The extent was made to100 ml with the equal solvent. This was taken as stock answer. similarly, 1.5 ml of above stock answer became diluted to 10ml with the diluent to get final attention of 75µg/ml.

Sample Solution Preparation

Weight equivalent to 50 mg of DUTA and TAM Hcl pattern had been weighed this was taken right into a 100 ml smooth dry volumetric flask and approximately 70ml of diluent turned into added and sonicated to dissolve it absolutely and extent made up to the mark with the same solvent. This became taken as stock solution. Similarly, 1.5 ml of above inventory answer was diluted to 10ml with diluent to get very last attention of seventy fiveµg/ml.

Intermediate Precision/Ruggedness

75 µg/ml of the above sample solution changed into injected 5 times in five extraordinary days and top areas had been recorded.

ACCURACY

For accuracy determination, 3 different concentrations had been prepared one by one i.e. 50%, 100% and a hundred and fifty% for the analyte and chromatograms are recorded for the identical.

Preparation of Sample solutions Preparation of 50% solution (37.5 µg/ml)

Approximately 25mg of DUTA and TAM were weighed and transferred to 100ml volumetric flask, it turned into dissolved with diluent and the volume turned into made upto the mark with identical solvent. similarly 1.5 ml of above solution changed into dilutedto 10ml with the diluent to get 37.5µg/ml.

- **Preparation of 100% solution (75 µg/ml)**

About 50mg of DUTA and TAM were weighed and transferred to 100ml volumetric flask, it turned into dissolved with diluent and the extent changed into madeon top of things with equal solvent. In addition 1.five ml of above solution changed into diluted to 10ml with the diluent to get seventy five µg/ml.

- **Preparation of 150% solution (112.5 µg/ml)**

About 75mg of DUTA and TAM were weighed and transferred to 100ml volumetric flask, it was dissolved with diluent and the volume was made up to the mark with same solvent. Further 1.5 ml of above solution was diluted to 10ml with the diluent to get 112.5µg/ml.

These solutions were filtered through 0.45µ membrane and stored. Each solution was injected three times under optimized conditions.

The % Assay was calculated using the following formula

$$\%\text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = average area counts of sample preparation. AS = average area counts of standard preparation. WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LINEARITY

For determination of linearity five different concentrations i.e. 25%, 50%, 100%, 125%, 150% were prepared and chromatograms are recorded for same.

Preparation of sample stock solution

Weight equivalent to 50 mg of sample was weighed in to 100ml volumetric flask, it was dissolved with diluent and the volume was made up to the mark with same diluent (500 µg/ml).

Preparation of 25% solution (25µg/ml)

0.5ml of stock solution was diluted to 10 ml with the diluent.

Preparation of 50% solution (50µg/ml)

1.0ml of stock solution was diluted to 10 ml with the diluent.

Preparation of 100% solution (75µg/ml)

1.5ml of stock solution was diluted to 10 ml with the diluent.

Preparation of 125% solution (100µg/ml)

2.0ml of stock solution was diluted to 10 ml with the diluent.

Preparation 150% solution (125µg/ml)

2.5ml of stock solution was diluted to 10 ml with the diluent.

10µl of each 25%, 50%, 75%, 100%, and 125% were injected in triplicate and recordedthe peak response.

5.1.1 LIMIT OF DETECTION (LOD)

Limit of detection is the lowest concentration of the substance that can be detected, not necessarily quantified by the method. (Regression statistics)

The minimum concentration at which the analyte can be detected is determined from the linearity curve by applying the following formula.

$$\text{Limit of detection (LOD)} = 3.3 \frac{\sigma}{S}$$

Where S – slope of the calibration curve σ – Residual standard deviation

LIMIT OF QUANTITATION (LOQ)

Limit of quantitation is the lowest concentration of the substance that can be estimated quantitatively. It can be determined from linearity curve by applying the following formula

$$\text{Limit of quantitation (LOQ)} = \frac{\sigma}{S} 10$$

Where S – slope of the calibration curveσ – Residual standard deviation

5.1.2 ROBUSTNESS

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Preparation of sample solution (75µg/ml)

About 50mg of DUTA and TAM were weighed and transferred to 100ml volumetric flask, it was dissolved with diluent and the volume was made up to the mark with same solvent. Further 1.5 ml of above solution was diluted to 10ml with the diluent to get 75µg/ml.

Effect of Variation of flow

The sample was analyzed at 0.7 ml/min and 0.9 ml/min instead of 0.8 ml/min, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of

mobile phase i.e. phosphate buffer: acetonitrile was taken in the ratio 15: 85 v/v and 25:75 v/v instead of 20:80 v/v, remaining conditionsare same. 10µl of the above sample was injected twice and chromatograms were recorded.

STABILITY STUDIES

Acid degradation

As it should be weighed and transferred 10mg of DUTA and TAM Hcl working standard right into a 10ml volumetric flask delivered about three ml of 0.1N HCl and sonicated for 10minutes and stored it in darkness for 24 hours then refluxed under heat at 60 degrees in a heating mantle for 1 hours. After one hour the sample answer changed into neutralized the use of 0.1N NaOH and diluted up to speed with diluent. This became taken as stock answer.

in addition 0.1 ml of the above stock solution changed into diluted to 10ml with diluent. mixed properly and filtered via zero.forty fiveµm filter. This solution changed into injected and analysed. top reaction was measured.

Alkaline Degradation

Appropriately weighed and transferred 10mg of DUTA and TAM Hcl running preferred into a 10ml volumetric flask .approximately 3ml of 0.1N NaOH turned into introduced and sonicated for 10minutes stored in darkness for twenty-four hours and refluxed under heat at 60 degrees in a heating mantle for 1 hour. After one hour the pattern answer changed into neutralized using zero.1N HCl and diluted up to speed with diluents. This was taken as inventory answer.

Similarly 0.1 ml of the above inventory solution became diluted to 10ml with diluent. Combined nicely and filtered via zero.45µm filter. This solution was injected andanalysed. Height response was measured.

Thermal Degradation

As it should be weighed and transferred 10mg of DUTA and TAM Hcl working preferred right into a 10ml volumetric flask and kept in oven beneath heat at one hundred and five ranges for twenty-four hours. This changed into taken as inventory solution.

similarly 0.1 ml of the above stock solution was diluted to 10ml with diluent. combined nicely and filtered thru 0.forty fiveµm filter. This answer turned into injected and analysed. height response become measured.

Peroxide Degradation

Accurately weighed and transferred 10mg of DUTA and TAM Hcl operating popular into a 10ml volumetric flask added approximately 3ml of 3% Hydrogen Peroxide (H2O2)and sonicated for 10 mins and saved in darkness for 12 hours and refluxed beneath warmness at 60 degrees in a heating mantle for 1 hours.

Similarly zero.1 ml of the above inventory answer changed into diluted to 10ml with diluent. mixed nicely and filtered through 0.forty fiveµm clear out. This solution become injected and analysed. peak reaction became measured.

Conclusion

In the modern pharmaceutical industry, HPLC is a major analytical tool applied at all stages of drug discovery, development and production. Fast and effective development of rugged analytical HPLC methods is more efficiently undertaken with a thorough understanding of HPLC principles, theory and instrumentation.

Liquid Chromatography (LC), which is one of the forms of Chromatography, is an analytical technique that is used to

separate a mixture in solution into its individual components. The separation relies on the use of two different "phases" or "immiscible layers," one of which is held stationary while the other moves over it. The separation occurs because, under an optimum set of conditions, each component in a mixture will interact with the two phases differently relative to the other components in the mixture. HPLC is the term used to describe Liquid Chromatography in which the liquid mobile phase is mechanically pumped through a column that contains the stationary phase.

Reference:

1. *D.V.Subba rao and P.Radhakrishnanad, " Stress degradation studies on Dutasteride and development of a stability indicating HPLC assay method for bulk drug and pharmaceutical dosage form." Chromatograpia 2008, 67, 841-845.*
2. *Pekka Keski-Rahkonen , Olavi Pärssinen, Esa Leppänen, Timo Mauriala, Marko Lehtonen and Seppo Auriola "Determination of tamsulosin in human aqueous humor and serum by liquid chromatography–electrospray ionization tandem mass spectrometry" Journal of pharmaceutical and biomedical analysis, 2007, 42(2), 606-612.*
3. *N. V. S. Ramakrishna, K. N. Vishwottam, S. Manoj, M. Koteshwara, S. Wishu, and D. P. Varma "Rapid, simple and highly sensitive LC-ESI-MS/MS method for the quantification of tamsulosin in human plasma". Biomedical chromatography, 2005,19(10) , 709–19.*
4. *Peng Wang and Lihe Liu "Determination of tamsulosin in dog plasma by liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry" Journal of chromatography B: Biomedical sciences and applications, 2004, 805(1), 7-11.*
5. *N. V. S. Ramakrishna, K. N. Vishwottam, S. Puran, S. Manoj, M. Santosh, S. Wishu, M. Koteshwara, J. Chidambara, B. Gopinadh and B. Sumatha, "Liquid chromatography–negative ion electrospray tandem mass spectrometry method for the quantification of tacrolimus in human plasma and its bioanalytical applications" Journal of chromatography B: Biomedical sciences and applications, 2004, 805(1), 13-20.*
6. *Zhefeng Zhang, Gengliang Yang, Guijian Liang, Haiyan Liu and Yi Chen, "Chiral separation of Tamsulosin isomers by HPLC using cellulose Tris (3,5-dimethhylphenylcarbamate) as a chiral stationary phase" Journal of Pharmaceutical and Biomedical Analysis, 2004, 34 (3), 689-693.*
7. *Hiroshi Matsushima, Ken-ichi Takanuki, Hidetaka Kamimura, Takashi Watanabe and Saburo Higuchi, "Highly sensitive method for the determination of tamsulosin hydrochloride in human plasma dialysate, plasma and urine by high-performance liquid chromatography–electrospray tandem mass spectrometry" Journal of chromatography B: Biomedical Sciences and Applications, 1997,695(2), 317-327.*