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Determination of Dutasteride from its Bulk Drug and Pharmaceutical Preparations by High Performance Thin Layer Chromatography

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A rapid, simple and sensitive high-performance thin layer chromatographic method (HPTLC) has been developed to assay dutasteride in capsules. The HPTLC analysis used a normal phase (silica gel 60 F254) as a stationary phase and a mobile phase consisting of mixture of acetonitrile:glacial acetic acid with UV detection at 210 nm. The validation data showed that the assay is sensitive, specific and reproducible for determination of dutasteride in this dosage form. Calibration curves were linear from 50-500 μg mL^{-1} ($R^2 > 0.998$). The accuracy of the method ranged from 99.17 to 99.94 %. Mean inter- and intra-assay relative standard deviations (RSD) were less than 2 %. The proposed method provided an accurate and precise analysis of dutasteride in its pharmaceutical preparations.

Key Words: HPTLC, Dutasteride, Silica gel, Densitometry.

INTRODUCTION

Dutasteride is a synthetic 4-azasteroid compound *i.e.*, a selective inhibitor of both the type 1 and type 2 isoforms of steroid 5a-reductase (5AR), an intracellular enzyme that converts testosterone to 5a-dihydrotestosterone (DHT). Dutasteride is chemically designated as (5a-, 17ß)-N-{2,5-*bis*-(trifluoromethyl)(phenyl}-3-oxo-4-azaandrost-l-ene-17-carboxamide[1,2].

Dutasteride inhibits the conversion of testosterone to 5-dihydrotestosterone (DHT)[3]. DHT is the androgen primarily responsible for the initial development and subsequent enlargement of the prostate gland. DHT is converted from testosterone by the enzyme 5α-reductase, which exists as two isoforms, types 1 and 2. In type 1,5α-reductase is found primarily in the skin and liver, but has also been found in prostatic tissue in BPH. While in Type 2,5α-dutasteride reaches peak serum concentrations *ca.* 2-3 h after being taken orally. Single doses of dutasteride 0.1-40 mg resulted in C_{max} values[4] of 0.6-166 ng/mL. DHT levels were significantly reduced from baseline by 72-95 % following single-dose administration of dutasteride 1-40 mg in volunteers (all $p \leq 0.001$ *versus* placebo). It is about 60 % bioavailable after oral administration and is primarily metabolized in the

liver (by CYP3A4 isoenzyme) and excreted in the feces. The drug has a large volume of distribution and is extensively distributed into central and peripheral compartments, including semen[4,5].

The mean semen dutasteride concentration was 3.4 ng/mL range 0.4-14 ng/mL) following 12 months of dutasteride 0.5 mg/d. On average, 11.5 % of the serum dutasteride concentration was found in the semen after 12 months. The terminal elimination half-life is 3-5 weeks and the drug remains detectable in serum for 4-6 months after treatment is discontinued[4].

Literature revealed that no high performance thin layer chromatographic method was available for the determination of dutasteride from bulk drug and its pharmaceutical preparations. Therefore a fast, economical, precise and accurate HPTLC method was developed for the determination of dutasteride from its bulk drug and pharmaceutical preparations.

EXPERIMENTAL

The formulation was purchased from market; standards were from reputed research centers. Acetonitrile, toluene and glacial acetic acid were from Qualigens. All dilutions were performed in standard volumetric flasks.

Standard stock solution was prepared by weighing 99.84 % pure dutasteride (25 mg) into a 25 mL volumetric flask, dissolving in methanol to get 1 mg/mL of dutasteride.

Chromatography

Procedure: Chromatography was performed on pre-coated silica gel 60 F 254 HPTLC plates (Merck). Before use they were pre-washed with methanol and dried in an oven at 110 ºC for 1 h. Samples (10 µL) were spotted 20 mm from the edge of the plates by means of a Camag Linomat IV sample applicator and the plates were developed to a distance of 90 mm in a Camag twin-trough chamber previously equilibrated with mobile phase toluene:ethyl acetate:acetic acid (7:3:0.5 by volume). The chromatographic conditions were optimized to achieve the best resolution and peak shape. Plates were evaluated by densitometry at $\lambda = 210$ nm with a Camag Scanner II, in conjunction with CATS software for quantitation. The wavelength used for densitometry was selected after acquiring *in situ* UV spectra of the drug.

Linearity of detector response: Solution containing dutasteride seven different concentrations was prepared in acetonitrile and water. Each of these solutions (10 mL) was applied to a plate, the plate was developed and the detector response to the different concentrations was measured. The drug peak area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area. The plot was linear for dutasteride in the concentration range 50-500 µg. This experiment was

carried out thrice and the mean was used for the calculations. The data were analyzed by linear regression least-squares fitting[6,7]. The statistical data obtained are given in Table-1.

TABLE-1
ANALYTICAL PERFORMANCE DATA

	Dutasteride
Linear working range (LWR) [µg]	50-500
Slope (m)	0.028
Intercept (b)	+3.3243
Correlation coefficient (R)	0.998

Assay

Pharmaceutical preparation: Twenty capsules of dutasteride were accurately weighed and the average weight of one capsule (555.23 mg) was calculated. Weighed capsules were first emptied and transferred to a 10 mL volumetric flask. The contents were dissolved in a minimum quantity of methanol and diluted upto the mark with methanol. To it 1 mL of internal standard was added, flask was sonicated for 5 min. This solution was spotted then the peak area responses for the drug and internal standard were measured also the retardation factor of the drug and internal standard was calculated at a wavelength of 210 nm. The densitograms were recorded and the peak area ratios of the drug to internal standard were calculated. A comparison of these peak area values with those obtained from the standard dutasteride was made and after applying the appropriate dilution factor, the amounts of dutasteride present was calculated.

From the bulk drug: Accurately weighed 10 mg of bulk drug of dutasteride and the above-mentioned procedure was applied. The results of assay are tabulated in Table-2.

TABLE-2
ASSAY EXPERIMENT

From pharmaceutical preparations			From bulk drug		
Weight of sample taken (mg)	Amount found (mg)	Assay (%)	Weight of sample taken (mg)	Amount found (mg)	Assay (%)
555.23	0.513	99.58	0.502	0.500	99.60
555.50	0.504	99.68	0.511	0.505	98.83
555.64	0.505	99.45	0.504	0.490	97.22
556.59	0.498	100.01	0.506	0.500	98.81
555.14	0.509	100.54	0.509	0.499	98.04
554.98	0.495	100.06	0.495	0.498	100.61
556.32	0.510	100.41	0.505	0.495	98.02
Mean	0.505	99.64	0.505	0.498	98.73
Coefficient of variation (%)	1.290	0.420	1.030	0.940	1.140

RESULTS AND DISCUSSION

Use of pre-coated silica gel HPTLC plates with toluene:ethyl acetate: acetic acid (7:3:0.5, by volume) resulted in good separation of the drug.

Regression analysis of the calibration data for dutasteride showed that the dependent variable (peak area) and the independent variable (concentration) were represented by the equations $Y = 0.028X + 3.3243$. The correlation of coefficient obtained was 0.998.

The system suitability experiment was carried out before the determination of dutasteride in unknown samples. The coefficient of variation was less than 2 % for replicate measurements of the same sample. This shows that the method and the system both are suitable for the determination of unknown samples.

The precision studies including the instrument precision, intra-assay precision and intermediate precision was carried out to evaluate the precision of the method. The intermediate precision included analysis on a different day and by a different analyst. The values of standard deviation and coefficient of variation were calculated. The standard deviations for intra assay precision was in the range of 0.10 to 0.31 and for inter assay precision it was 0.27 to 0.72. The coefficient of variation was in the range of 0.06 to 0.09 for intraday and for interday they were 0.06 to 0.48. The low values of standard deviation and coefficient of variation indicate high precision of the method (Table-3).

TABLE-3
PRECISION EXPERIMENT

Obs. No.	Conc. of drug (μg)	Day to day comparison			Analyst to analyst comparison		
		Mean	SD	COV (%)	Mean	SD	COV (%)
1	150.0	150.21	0.10	0.06	150.41	0.72	0.48
2	300.0	300.46	0.26	0.09	300.41	0.40	0.13
3	450.0	450.40	0.31	0.07	450.41	0.27	0.06

The accuracy of the experiment was established by spiking pre-analyzed sample with known amounts of the drugs at three different concentration levels *i.e.* 80, 100 and 120 % of the drug in the capsule (the external standard addition technique). The spiked samples were then analyzed for seven times. The results from recovery analysis are given in Table-4. The mean recovery is within acceptable limits, indicating the method is accurate.

To study the specificity of the method for the determination of dutasteride, the pure standard was subjected to various stress conditions like heat, light, oxidation and hydrolysis. The specificity of the method was demonstrated by the separation of the probable impurities and probable degradation products from the main peak of dutasteride.

TABLE-4
ACCURACY AND RECOVERY ANALYSIS

Set no.	Dutasteride (0.5 mg) Mean assay		
	Amount of drug added	Amount of drug found	Recovery [%]
SET 1 (80 %)	0.41	0.92	99.33
SET 2 (100 %)	0.50	1.04	99.41
SET 3 (120 %)	0.61	1.12	99.47

The robustness of the method was studied simultaneously along with the method development process.

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