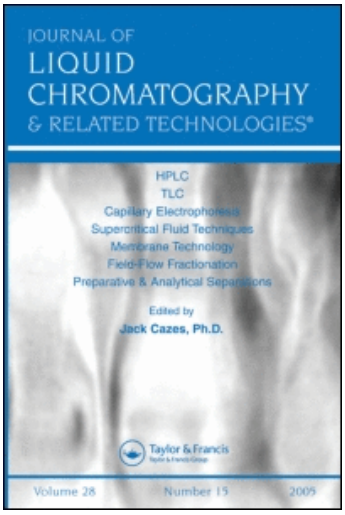

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A STABILITY-INDICATING HPLC METHOD TO DETERMINE FINASTERIDE IN A TABLET FORMULATION

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ABSTRACT

A simple high performance liquid chromatographic method was developed for simultaneous determination of finasteride and its degradation products. HPLC analysis was carried out using a C_{18} column and methanol : water (70 : 30) as the mobile phase. Detection was carried out at 210 nm using a flow rate of 1.0 mL per min. Finasteride was eluted at 6 min. Standard deviation values were below 2%.

The method was validated as stability-indicating by forced decomposition of finasteride using acid, base, hydrogen peroxide, heat, and light. Chromatograms showed good resolution, sensitivity, and no interference of degradation products. The response was linear over the concentration range of 50 to 800 μg/mL, with correlation coefficients of variation greater than 0.9995. Recovery studies gave results between 100 to 103%.

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INTRODUCTION

Finasteride (*N*-tert-Butyl-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide) (Fig. 1) is an azasteroid that inhibits 5α-reductase, the enzyme responsible for conversion of testosterone to the more active dihydrotestosterone, and therefore, has anti-androgenic properties. It is formulated in tablets in a dose of 5 mg daily for the management of benign prostatic hyperplasia.

We developed a simple, rapid, reproducible, economic, and stability-indicating HPLC method for the simultaneous determination of finasteride in the presence of its degradation products. Some analytical methods for the analysis of finasteride in biological fluids have been described.[1–7]

Different HPLC conditions for the evaluation of finasteride as drug substance and drug product are described.[8] These methods use different columns, mobiles phase, wavelength detection, and heat.

The method reported here for the determination of finasteride in a pharmaceutical formulation was validated following the analytical performance parameters suggested by ICH.[9]

EXPERIMENTAL

Materials and Reagents

The working standard finasteride was developed locally using a crystallizing technique. Solvents were HPLC grade. Water HPLC grade was obtained by distillation and passed through a 0.45 micron membrane filter.

A commercial local tablet formulation was studied. Its composition was finasteride 5 mg, in a matrix of microcrystalline cellulose, starch, povidone, lactose, talc, sodium starch glycolate, colloidal silicon dioxide, and magnesium stearate.

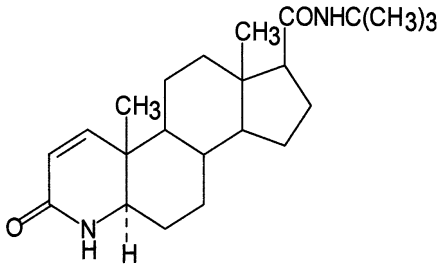

Figure 1. Finasteride structure.

Instrumentation

The HPLC system consisted of a dual piston reciprocating pump Spectra Physics (Model ISO Chrom. LC pump), a detector UV-Vis Hewlett Packard (Model 1050), an integrator Hewlett Packard (Series 3395), and a Rheodyne injector (Model 7125).

HPLC Conditions

The experiment was performed on a LiChroCART® 250∗4 mm HPLC Cartridge LiChrospher® 100 RP-18 (5 μm) Merck (Darmstadt, Germany). The finasteride working standard solution was analyzed at different mobiles phase proportions and retention time and tailing factor were evaluated.

The mobile phase consisted of methanol : water (70 : 30, v/v) filtered and degassed under reduced pressure prior to use. Separation was carried out isocratically at room temperature ($20 \pm 2^{\circ}C$) and a flow rate of 1.0 mL/min, with UV detection at 210 nm. Detector sensitivity was set at 2 a.u.f.s. The volume of each injection was 20 μL. In these conditions, finasteride retention time was roughly 6 min.

Procedure

Solutions were prepared on a weight basis and volumetric flasks used as suitable containers in order to minimize solvent evaporation.

Prior to injecting solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system.

Quantitation was accomplished using an external standard method.

System Suitability

The chromatographic system was in agreement with the following parameters, calculated from six injections of a freshly prepared resolution test mixture: minimum of theoretical plates in the chromatographic column greater than 1240 (plates/meter), calculated on the basis of finasteride peak; the relative standard deviation (RSD) of finasteride peak areas was 0.23% and tailing factor less than 1.1.[10]

Preparation of the Solutions

Working Standard Solution

Fifty milligrams of finasteride were taken in a 100 mL volumetric flask, dissolved in 40 mL of mobile phase, sonicated for about 5 min, and then diluted to volume with mobile phase.

Precision

Repeatability studies were done by assaying six sample preparations of a single lot of tablets.

Twenty tablets were weighed and finely powdered and an accurately weighed powder sample equivalent to one tablet was placed in a 10 mL volumetric flask. Eight milliliters of mobile phase was added and the flask was kept in an ultrasonic bath for 5 min. The mixture was then diluted to 10 mL with mobile phase, thoroughly mixed, and filtered through a Whatman No 42 paper.

Intermediate precision was evaluated by comparing the results obtained of two repeatability assays by two analysts.

Calibration Solutions

Six solutions were prepared in mobile phase at concentrations ranging from 50 μg/mL to 800 μg/mL.

Selectivity

Method selectivity was determined by degrading finasteride as follows:

Finasteride was subjected to thermal (in an open container, in an oven at 110°C for 24 hr.) and photochemical degradation (in an open container exposed to daylight for 24 hr.)

Fifty milligrams of finasteride were dissolved in 10 mL of: water, HCl 1 N, NaOH 1 N, and H_2O_2 100 vol, refluxed for at least 30 min. and degradation was monitored as a function of time. Each solution was neutralized and suitably diluted with mobile phase in a 100 mL volumetric flask.

Accuracy

The accuracy of the assay was assessed at 80, 100, and 120% of finasteride by recovery experiments, using tablets from the same lot of a commercial formulation.

Twenty tablets were weighed and finely powdered and an accurately weighed powder sample equivalent to four, five, and six tablets was placed in a 25 mL volumetric flask. Twenty milliliters of mobile phase was added and the flask was kept in an ultrasonic bath for 5 min. The mixture was then diluted to 25 mL with mobile phase, thoroughly mixed, and filtered through a Whatman No 42 paper.

RESULTS AND DISCUSSION

Method Development

The effect on retention time and tailing factor was evaluated by changing the mobile phase proportion (Table 1).

Precision

Precision was considered at two levels of ICH suggestions: repeatability and intermediate precision. Repeatability was evaluated by analyzing six sample preparations of a single lot of tablets of finasteride giving a RSD less than 1.5% and minimal variation in retention time.

Intermediate precision was determined by carrying out two precision assays of one lot of commercial formulations. For each precision assay the results were as follows: mean values 95.43 and 97.33%, standard deviations 1.22 and 0.63, and RSD 1.3% and 0.6%. Test "t" comparing two sample means with 99.9% confidence for 10 degrees of freedom, disclosed that both results were not significantly different *inter se* (t_{n-2}, α: 0.001) = 4.587 (Table 2).

Selectivity

Neither formulation ingredients nor degradation products interfered with quantitation of finasteride.

Table 1. Effect of Mobile Phase Composition on Retention Time and Tailing Factor

Mobile Phase	Finsteride Retention Time (min)	Tailing Factor
Methanol : Water (60 : 40)	15.8	2.5
Methanol : Water (80 : 20)	3.8	1.1

Table 2. Assay Precision

	Analyst 1			Analyst 2		
Sample No.	mg/ Tablet	Percentage	RSD (%)	mg/ Tablet	Percentage	RSD (%)
1	4.69	93.70	0.3	4.82	96.42	0.9
2	4.74	94.77	0.8	4.85	97.06	0.7
3	4.88	97.47	0.3	4.84	96.92	0.7
4	4.74	94.76	0.3	4.88	97.71	0.7
5	4.80	96.09	0.3	4.89	97.76	0.9
6	4.79	97.76	0.2	4.90	98.08	0.8
Means	4.77	95.43	1.3	4.86	97.33	0.6

RSD, relative standard deviation.

All samples were analyzed using the assay chromatographic conditions described. No evidence of interactive degradation products was seen during evaluation.

However, finasteride showed degradation products after alkaline and acid hydrolysis, oxidation, and heat-dry (Table 3).

Selectivity was demonstrated showing that finasteride peaks were free of interference from degradation products, indicating that the proposed method can be used in stability assay (Fig. 2).

Linearity

Curves of peak areas vs. concentration proved linear (Fig. 3).

Table 3. Selectivity: Degradation Conditions of Finasteride

Condition	Time (hs)	% Recovered	RRT of Degradation Products
Heat dry, 110°C	24	97.6	1.92, 2.05
Water, refl.	0.5	92.9	None detected
Day light	24	95.2	None detected
Acid 1 N HCl, refl.	0.5	94.1	0.40, 0.42, 0.52, 0.74, 0.87
Base 1 N NaOH refl.	0.5	95.3	0.40, 0.42, 0.52, 0.58, 0.74
Hydrogen peroxide 100 vol, refl.	0.5	94.7	0.28, 0.41, 0.67

Ref, refluxed.
RRT, relative retention time.

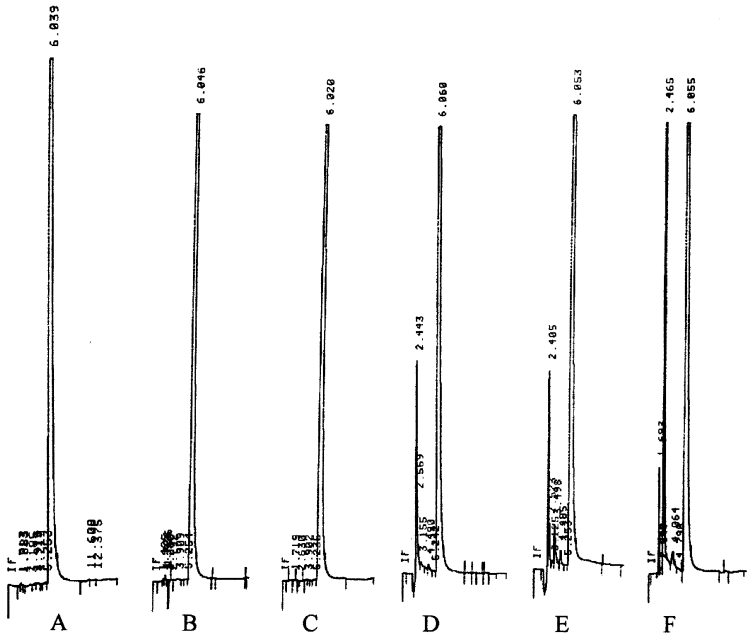

Figure 2. Chromatograms of finasteride with its potential related substances. Peak identities: (A) chromatogram of heat dry degradation; (B) chromatogram of water degradation; (C) chromatogram of photolytic degradation; (D) chromatogram of acid degradation; (E) chromatogram of alkaline degradation; (F) chromatogram of oxidative degradation.

The linearity of finasteride peak area responses was demonstrated from approximately 10 to 160% of the 0.5 mg/mL working analytical concentration by a correlation coefficient greater than 0.9995 (Table 4).

Accuracy

According to recovery studies performed in 80, 100, 120% of the analytical concentration, the extraction of the active component was shown to be quantitative (Table 5).

Method accuracy was demonstrated by plotting the amount recovered vs. the amount added (in mg). Linear least squares analysis of the data yielded a correlation coefficient (r) value and slope of 0.9992 and 1.0339 respectively. The r value indicates that the method is linear over the concentration range investigated. The slope value was close to unity and the intercept was not

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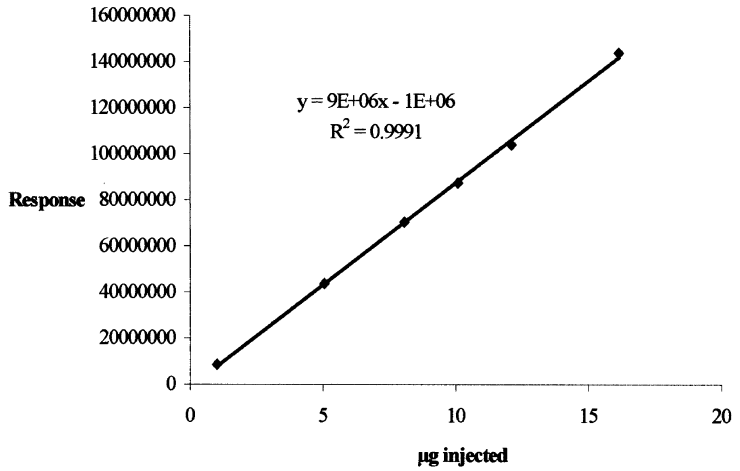

Figure 3. Linearity.

significantly different from zero (t test, $p = 0.05$), which confirmed the accuracy of the method over the range investigated.

Since the results obtained were within the acceptable ±3% range, the method was deemed to be accurate.

Table 4. Linearity Data

% w/w	Injected (µg)	Average Peak Area Response	RSD (%)
10	1.01	8613929	0.06
50	5.05	43621664	0.33
80	8.08	70361920	0.06
100	10.1	87346475	0.10
120	12.12	103940333	0.14
160	16.16	143871580	0.80
Slope[a]		8847604 ± 372567	1.5
Intercept[b]		−1153377 ± 3731438	

Finasteride: $Y = 8.85 \times 10^6 X - 1.15 \times 10^6$.
[a]Confidence limits of the slope ($p = 0.05$).
[b]Confidence limits of the intercept ($p = 0.05$).
RSD, relative standard deviation.

Table 5. Results of the Recovery Analysis

% w/w	Amount of Drug Present (mg)	Amount Found (mg per Tablet)	Recovered (%)	Average Recovered ($n=3$)	RSD ($n=3$)
80	20.4	20.6	100.9		
	20.3	20.6	101.3	101.3	0.3
	20.5	20.8	101.6		
100	24.6	24.8	101.0		
	24.5	24.5	100.0	100.9	0.8
	26.0	26.4	101.6		
120	29.6	30.4	102.9		
	30.7	31.3	102.1	102.1	0.7
	31.0	31.4	101.4		
Overall Recovery ($n=9$)		101.4	0.6		

RSD, relative standard deviation.

CONCLUSION

The assay procedure has been successfully applied to the study of finasteride in tablets and may be used as a stability-indicating method. The results of validation showed that the method was unaffected by assay time.

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