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Short communication

LC determination of finasteride and its application to storage stability studies

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Abstract

The development of a simple, sensitive, rapid, and reproducible reversed-phase high-performance liquid chromatographic assay of finasteride (proscar) in preformulation, and its application to forced degradation studies has been carried out. The method showed excellent linearity ($r^2 \geq 0.9997$) in the range 20-600 μg ml^{-1} using a Shimpak C_8 column (5μm, 15.0 cm x 4.6 mm) and UV-detection (210 nm) at ambient temperature (25 $\pm 1^0$C) with a mobile phase of acetonitrile and water (95:05,v/v) and flow rate of 0.7 ml min^{-1}. All peaks are eluted in $<$ 10 min and the method has good precision. This method showed good efficiency for the analysis of forced degraded samples, studied at different temperatures and humidities. The results manifest that the shelf-life of proscar is greater than two years at room temperature, under proper storage conditions.

Keywords: Proscar; Finasteride; HPLC; Stability; 5-α Reductase inhibitor

1. Introduction

Finasteride [Proscar, *N*-(1,1-dimethylethyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide (Fig. 1) is a 4-aza-3-oxosteroidal inhibitor of human 5α-reductase [1–3]. It is a member of the family of compounds referred to as 4-azasteroids. Its synthesis has been described [4]; the compound appears to have some potential as a therapeutic agent for benign prostatic hyperplasia [5]. The 4-azasteriods are a newly developed family of compounds that block the intracellular metabolism of testosterone and thereby enable the more potent androgen dihydrotestosterone to come into play [1–3].

In recent years, stringent quality control in the pharmaceutical industries has given rise to a growing need for simple, selective and sensitive analytical methods for the study of the degradation products as well as of the impurities, and thereby to assure the quality of the drug. High-performance liquid chromatography (HPLC) is a

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method with numerous applications for the quantitation of drugs in various matrices-preformulations, dosage forms, and in biological fluids.

A survey of the literature reveals that no analytical method has been reported in the literature for the determination of finesteride (proscar) in the preformualtion stage, nor in the form of a formulation, and no stability studies have been carried out on this important non-pharamacopoeial drug. A few papers have been published [6–9] relating to in vitro determination of proscar and its metabolites in biological fluids using HPLC.

The objective of this study was to develop a simple, sensitive, rapid and reproducible HPLC method for the determination of proscar in preformulation in the presence of impurities and or degradation products. The stability studies were performed under forced degradation conditions, viz., elevated temperatures at 49 ± 1°C for 180 days, and 75 ± 5% relative humidity (at 45°C) for 180 days. Studies were also carried out under proper storage conditions for 2 years.

2. Experimental

2.1. Reagents

Proscar procured from M/s Recon Ltd. (Bangalore, India) was used as received. Acetonitrile and water (Merck, India) were of HPLC grade.

2.2. Instrumentation and chromatographic conditions

Chromatographic analysis were carried out using Shimadzu, Japan HPLC system with LC-10 AD solvent delivery units, and SPD-10 A UV/VIS detector set at 210 nm. Chromatograms were recorded and processed on CR-4A Chromatopac data processor and Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA) with a 20 μl fixed loop.

Chromatographic separation was accomplished using a Shimpac C_8 column (5 μm, 15.0 cm × 4.6 mm). The separation of proscar from its impurities and degradation products was achieved using acetonitrile and water (95:05, v/v) as the eluent, pumped at a flow rate of 0.7 ml min^{-1}. The eluent was filtered (pore size, 0.45 μm) before use and then degassed by sonication in an ultrasound bath. The assays were performed at ambient temperature (25 ± 1°C).

2.2.1. Standards and sample preparation

Stock solutions of standards for HPLC were prepared by dissolving 25 mg proscar in the mobile phase, and making-up the solution to 50 ml. The working solutions were prepared by dilution of the stock solution, and all the solutions were stored at room temperature (25 ± 1°C). Quantifications were achieved by regression analysis of the peak areas against concentration; triplicate injections were made. Calibration curves were constructed, which were linear over the concentration range of 20–600 μg ml^{-1}.

2.2.2. Stability studies

US Food and Drug Administration (FDA) guidelines for current Good Manufacturing Practice (GMP) were followed [10,11]. The pure samples were packed in simulated packs and kept in a humidity chamber for a known period, at 75 ± 5% relative humidity and at 45 ± 1°C. In another experiment, the samples packed as described above were kept in a vacuum oven at 49 ± 1°C for the desired period. In a third experiment, the samples were kept under normal conditions with proper packing for a period of 2 years.

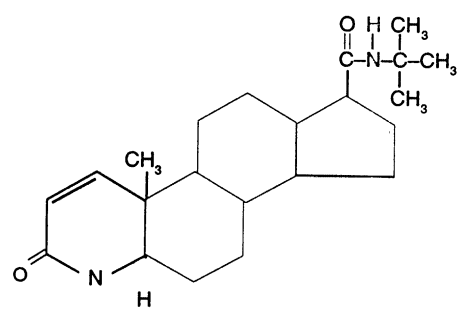

Fig. 1. Finasteride.

Table 1
Validation parameters of the proposed method

Parameters	Values[a]
Concentration range	20–600 mg l^{-1}
Recovery	99.69–100.06%
S.D.	0.01–0.07
R.S.D.	<0.2%
Linearity	0.9997
Intercept	0.1831
Slope	0.1400
Limit of Detection	0.4 ppm

[a] Average of five determinations.

3. Results

3.1. HPLC profile

3.1.1. Mobile phase

The solubility of proscar in solvents is in the order: acetonitrile > methanol > water. Earlier studies on the determination of proscar in biological matrices have reported the use of eluent, acetonitrile:methanol:water (26:39:35, v/v) [6] and (5:6:7, v/v) [7]. In our studies a combination of acetonitrile:water (95:05, v/v) has proved to be most efficient.

3.1.2. Stationary phase

Currently, the most widely used HPLC mode is that of reversed-phase high performance liquid chromatography (RP-HPLC) using a stationary phase chemically bonded with octyl silanes, which are the most commonly used modifiers of silica surface [12]. Hence, the choice of octylsilane as stationary phase is justified. Furthermore, the selection of an aqueous mobile phase, incorporating acetonitrile as organic modifier, has resulted in an excellent separation of proscar, which is an electrically uncharged species.

3.1.3. UV-detector

The UV spectrum of proscar in methanol indicated the presence of a strong absorption band with the maximum at 204 nm ($\varepsilon = 15900$ mol^{-1} cm^{-1}) and a shoulder (between 220 and 270 nm, $\varepsilon = 2400–600$) of much lower intensity than expected for the α, β-unsaturated ketone functionality ($\varepsilon = 10000–18000$, $\lambda_{max} = 230–270$ nm) unperturbed by nitrogen. Similar results have been reported earlier [7]. The absorption of proscar at longer wavelength was not of sufficient intensity for sensitive UV detection. Therefore, the detection wavelength of 210 nm ($\varepsilon = 14700$ mol^{-1} cm^{-1}) had to be chosen for proscar.

3.1.4. Linearity

Detector response for proscar was linear in the range 20–600 μg ml^{-1}. The resulting data was plotted as peak area (measured electronically by the integrator) versus concentration and studied by linear regression analysis. Linearity plots display correlation coefficient equal to 0.9997.

3.1.5. Validation parameters

Validation parameters were studied using simulated preparation with concentrations of 20, 40, 80, 100, 200, 400 and 600 μg ml^{-1}. Parameters for each concentration were calculated as average of five determinations. The details are shown in Table 1. For routine analysis 100 μg ml^{-1} concentration was employed.

3.1.6. Resolution

Good chromatographic resolution was observed using the above method. Fig. 2 shows the adequate separation of proscar from its degraded products. No further investigations on the characterization of degraded products were carried out.

3.1.7. Stability

Preformulation stability studies are usually the first quantitative assessment of the chemical stability of a new drug. Hence, stability of proscar, a new, non-pharmacopoeal drug, was evaluated under two types of accelerated conditions. The results are shown in Table 2. The results do not manifest any appreciable change in the potency of the drug, which indicates that the shelf-life of proscar is greater than 2 years at room temperature, under proper storage conditions. This conclusion has been drawn, because, first of all, the analysis was carried out periodically using HPLC; secondly, optical rotation studies have confirmed the HPLC results; and thirdly, the results are in

Table 2
Stability studies on proscar

Tests	Initial results	Accelerated stability study for[a]			Elevated temperature study for[b]	
		45 days	90 days	180 days	90 days	180 days
Description	Off-white crystalline powder	Off-white crystalline powder	Off-white crystalline powder	Off-white crystalline powder	Off-white crystalline powder	Off-white crystalline powder
Melting range (°C)	254–256	254–256	254–256	254–256	255–256	253–254
Specific optical rotation (°)	−11.5	−11.4	−11.4	−11.3	−11.2	−11.2
Assay (%)	100.03	100.00	99.98	99.30	98.70	98.60

[a] $75 \pm 5\%$ relative humidity and 45 ± 1°C.
[b] 49 ± 1°C in vacuum oven.

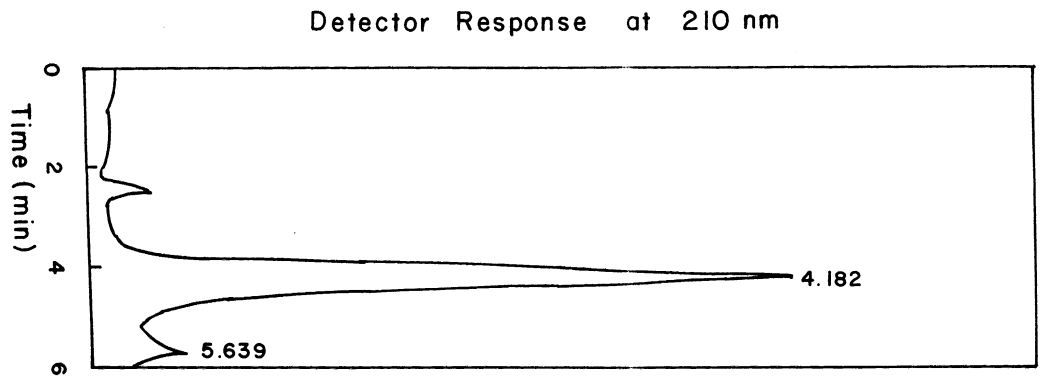

Fig. 2. HPLC chromatogram showing separation of finasteride from its degradation products.

agreement with the theoretical prediction based on physico-chemical principles [13]. All the data are in conformity with our observation, viz. that the potency of the drug is $>98\%$ after 2 years under normal storage conditions.

4. Conclusion

A simple and rapid procedure for HPLC analysis of proscar has been developed. This procedure is suitable for the assay of proscar in preformulation. The technique has been successfully employed for stability studies, which confirm that there is no loss in potency of drug for a period of 2 years.

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