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Short communication

Finasteride in biological fluids: extraction and separation by a graphitized carbon black cartridge and quantification by high-performance liquid chromatography

Giuseppe Carlucci*, Pietro Mazzeo

Dipartimento di Chimica, Ingegneria Chimica e Materiali, Università dell'Aquila, Via Vetoio, 67010 Coppito (L'Aquila), Italy

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Abstract

A simple, specific and sensitive high-performance liquid chromatographic method has been developed and validated for the determination of finasteride in human plasma. A solid-phase extraction procedure was used to isolate finasteride from the biological matrix before quantitative analysis. The analyte was separated on a Symmetry reversed-phase column using acetonitrile–0.04 *M* orthophosphoric acid (pH 4.0) as mobile phase and quantified by measuring its UV absorbance at 215 nm. The limit of detection for the analyte was 0.005 μg/ml. 4-Androstene-3,17-dione was used as internal standard. The calibration graph of the method was linear from 0.01 to 3.0 μg/ml of finasteride in human plasma, and the coefficient of variation less than 4.5%. This HPLC procedure is simple, precise and accurate for the determination of finasteride in human plasma.

Keywords: Finasteride

1. Introduction

Finasteride, or (5α,17β)-(1,1-dimethylethyl)-3-oxo-4-azaandrost-1-ene-17-carboxamide (Fig. 1A), is an orally active agent for the treatment of the benign prostatic hyperplasia. It is a competitive inhibitor of the steroid 5α-reductase, the enzyme that metabolizes testosterone in the androgen target organs to the more potent hormone 5α-dihydrotestosterone. Increased 5α-reductase activity and elevated dihydrotestosterone levels in the hyperplastic human prostate have been reported for animals and humans treated with finasteride [1–8].

Two analytical HPLC methods have been reported

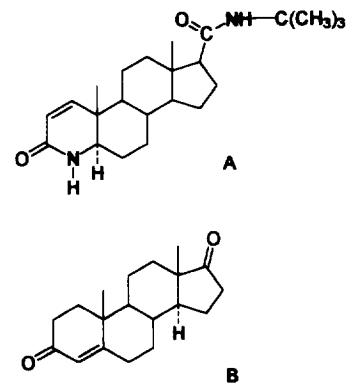

Fig. 1. Chemical structures of finasteride (A) and internal standard (B).

*Corresponding author.

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for assaying finasteride. One of them [9] uses a combination of two cartridges (Sep-Pak C18 and Sep-Pak-CN) for sample preparation, while in the other [10] only the Sep-Pak-CN is used. The use of a graphitized carbon black (GCB) cartridge for the determination of finasteride in biological fluids has not yet been described. These carbon-based extractions were all extremely efficient and provided purer extracts than those obtained with the other methods. The commercial extraction cartridges used in the present study contained non-porous carbon. With an adequate total surface area of 100 m^2/g these cartridges should be free of diffusion effects, which require slow and controlled flow-rates with porous GCB.

This paper describes the development of a HPLC method with UV detection for the determination of finasteride in human plasma. The procedure, based on the use of the high-performance liquid chromatography, allows accurate and precise results.

2. Experimental

2.1. Chemicals and materials

Finasteride was kindly supplied by Merck Sharp and Dohme (Italy). 4-Androstene-3,17-dione, used as internal standard, was purchased from Sigma (St. Louis, MO, USA). Chloroform was purchased from Aldrich (Milan, Italy). Acetonitrile and methanol (HPLC grade), and all other analytical-grade reagents were obtained from Farmitalia-Carlo Erba (Milan, Italy). Water (HPLC grade) was obtained by distillation in glass and passage through a Milli-Q water purification system (Millipore, Bedford, MA, USA). The extraction apparatus was a Supelco solid-phase extraction manifold equipped with a drying attachment (Supelco, Bellefonte, PA, USA). Alltech Carbograph SPE cartridges (300 mg, 6 ml) were purchased from Alltech Italia (Milan, Italy).

2.2. Chromatographic system and conditions

HPLC analysis was carried out using a Waters (Waters, Milford, MA, USA) system composed of the following: a Model 510 pump, a Model 486 LC variable wavelength absorbance detector connected to a Model HP 3396-II integrator (Hewlett-Packard, Rome, Italy). A Model 7125 sample injector (Rheodyne, Cotati, CA, USA) equipped with a 50-μl loop was used. The analysis was performed on an analytical 250×4.6 mm I.D. reversed-phase Symmetry (5 μm particle size) column (Waters), protected by a 20×4.6 mm I.D. disposable (40 μm particle size) Pelliguard precolumn (Supelco). Separations were performed at room temperature. The mobile phase consisted of a mixture of acetonitrile and 0.04 *M* orthophosphoric acid pH 4.0 (45:55, v/v). The acetonitrile was filtered through a FA 0.5-μm filter, while water was filtered through an HA 0.45-μm filter (Millipore). The mobile phase was prepared daily, sonicated before use and delivered at a flow-rate of 1.2 ml/min. Column eluate was monitored at 215 nm.

2.3. Standard solutions and calibration curves

Stock solutions of finasteride and 4-androstene-3,17-dione (internal standard) were prepared by dissolving 10 mg of each compound in 10 ml of methanol. These solutions could be stored at −20°C for over 1 month with no evidence of decomposition. The solution of internal standard obtained was diluted with methanol to obtain a concentration equivalent to 10 μg/ml. Standard solutions of the drug were prepared with control human plasma in the concentration range of 0.01–3.0 μg/ml. An aliquot of the internal standard solution was added to each sample to give an internal standard concentration equal to 1.0 μg/ml. The standards were treated in the same manner as the samples to be analysed. A calibration curve was obtained by plotting the peak-area ratio of the drug to internal standard, obtained after extraction, versus its concentration.

2.4. Sample preparation

Heparinized blood samples from various volunteers were centrifuged and the plasma was collected and frozen at −20°C until required for assay. Samples were thawed just before the extraction procedure, thoroughly agitated and centrifuged at 1500 *g* for 10 min. The graphitized carbon black cartridges were places in a Luer syringe that fitted the top of

the Supelco vacuum manifold, which may be loaded with up to twelve cartridges. A vacuum of 250–500 Torr was applied to the manifold to carry out the various steps of the extraction. A 2.0-ml rinse of HPLC-grade water followed by 4 ml of methanol served to desorb any organic impurities from the cartridges and to wet the packing before the introduction of the plasma sample. Then 1.0 ml of plasma sample was added with 100 μl of a 10.0 μg/ml solution of internal standard, briefly vortexed and passed through the cartridge, followed by 2 ml of water and 4 ml of a mixture methanol–water (20:80) and the eluate was discarded. A 2-ml volume of a mixture of chloroform–methanol (20:80, v/v) was then applied to the cartridge and the eluate collected. This fraction was finally centrifuged (1000 *g* for 10 min), filtered through a WTP 0.5 μm, evaporated to dryness with nitrogen stream under vacuum utilizing the Supelco drying attachment. The sample was then reconstituted at 1.0 ml with mobile phase and mixed with a vortex agitator. Aliquots of each sample (50 μl) were chromatographed using the apparatus described in Section 2.2.

2.5. Validation of the method

The precision and accuracy of the method were determined by preparing pools of plasma containing finasteride at two different concentrations (0.05 and 1.0 μg/ml). The values for finasteride concentration, for each standard concentration, were determined by nine repeated analyses, through regression analysis. The method was found to be reproducible and accurate. The coefficient of variation (within-day and between-day variability) for finasteride was less than 4.5%. The mean extraction efficiency, calculated by comparison of the peak-area ratios of the extracted samples with those of methanolic standards of same concentration, was 93%. Data obtained in different days were invariably found to be in good agreement. Results are given in Table 1. For testing the stability of the plasma samples has been carried out a comparison of the data obtained between fresh plasma samples and samples of same concentration frozen at −20°C for a period of one month. The results showed no differences.

3. Results and discussion

Fig. 2 shows a typical chromatogram of a drug-free plasma (A), a spiked plasma sample (B) and a 2 h after dose sample from a volunteer (C). No endogenous plasma components or metabolites were observed near the retention times corresponding to finasteride or internal standard. Retention times for finasteride and internal standard were 14.6 and 9.8 min, respectively. The calibration graph for finasteride was linear from 0.01 to 3.0 μg/ml. The validity of the liquid chromatographic assay was established, as previously reported, through a confirmatory study of sensitivity, accuracy and precision. The correlation coefficient was 0.9996. The straight line equation for finasteride was $y=1.48x-0.54$, where y is the peak-area ratio between finasteride and the internal standard in the arbitrary units of the HP-3396-II system used and x is the drug concentration (μg/ml). From this equation the concentration of the analyte can be determined. The lower limit of detection, defined as a signal-to-noise ratio of three, was 5 ng/ml. We have developed a simple, precise and accurate HPLC method for the determination of finasteride in plasma. The method described in this paper introduces a highly selective procedure of solid-phase extraction with graphitized

Table 1
Within-day and between-day variation for finasteride determination

Concentration (μg/ml)	Within-day		Between-day	
	Recovery±S.D. (%)	C.V. (%)	Recovery±S.D. (%)	C.V. (%)
0.05	92±4	4.3	90±4	4.4
1.0	94±0.5	0.5	92±0.5	0.5

Reported values are the mean of the nine repeated analyses for each day.
C.V.=coefficient of variation.

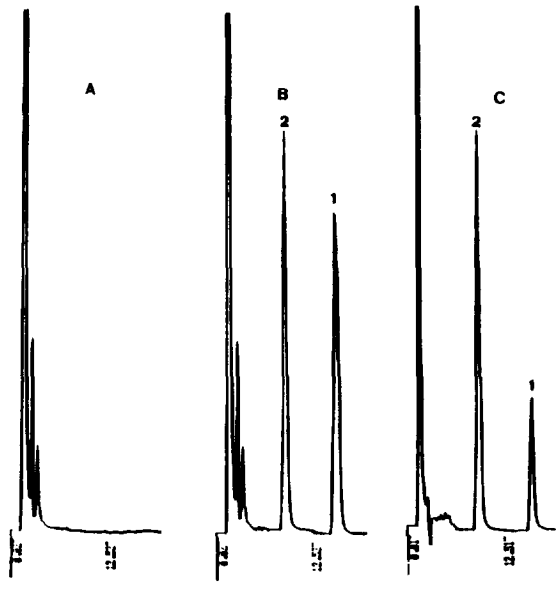

Fig. 2. HPLC profiles of human plasma extracts. (A) Drug-free human plasma; (B) drug-free human plasma spiked with 0.3 μg/ml finasteride (peak 1) and 1.0 μg/ml internal standard (peak 2); (C) plasma sample from a volunteer (2 h after a dose of 10 mg of drug) containing 0.12 μg/ml of finasteride. Column, Symmetry (250×4.6 mm I.D.); mobile phase, 0.04 *M* orthophosphoric acid (pH 4.0)–acetonitrile (45:55, v/v); UV detection at 215 nm.

carbon black cartridge utilizing isolation mechanisms achieving high recoveries.

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