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BIOMEDICAL APPLICATIONS

Synthesis of 5,6,6- $[^2\text{H}_3]$ finasteride and quantitative determination of finasteride in human plasma at picogram level by an isotope-dilution mass spectrometric method

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Abstract

Finasteride is a potent inhibitor of the enzyme steroid 5α -reductase now approved as a drug for the treatment of benign prostatic hyperplasia. We describe an original method for the quantitative determination of finasteride at picogram level in human plasma by isotope-dilution gas chromatography mass spectrometry. 5,6,6- $[^2\text{H}_3]$ Finasteride was synthesized with an high ratio of trideuteration (finasteride/ $[^2\text{H}_3]$ finasteride = 0.007) allowing its optimal use as internal standard. Plasma samples were purified in a single-step procedure on solid-phase extraction C_{18} columns with a recovery $\geq 90\%$. Samples were injected in the GC-MS instrument without any derivatization and the minimum detection level of finasteride was 50 pg with a signal-to-noise ratio of 6:1. The coefficients of variation for the 5 and 10 ng/ml (plasma) concentrations were 5.8% and 4%, respectively. The method has been applied to the determination of the plasma pharmacokinetic of finasteride in five male volunteers treated with a single 5-mg dose of the drug, affording kinetic parameters which are in good agreement with the values previously reported with a different methodology. The present method results accurate, specific, sensible and reliable for a routinely determination of finasteride at picogram levels.

Keywords: Isotope-dilution mass spectrometry; Finasteride

1. Introduction

Finasteride (N-*tert*-butyl-3-oxo-4-aza-androst-1-ene-17 β -carboxamide) is a 4-azasteroid synthesized by Merck Sharp & Dohme [1] that selectively inhibits steroid 5α -reductase, the enzyme responsible of the conversion of testosterone (T) into dihydrotestosterone (DHT). The

high inhibitory activity of finasteride towards the human prostatic isozyme and its low toxicity allowed its use as a drug for the treatment of DHT dependent pathologies. Several clinical studies have demonstrated the efficacy of finasteride in reducing the pathologic effects of benign prostatic hyperplasia (BPH) [2–4]. Finasteride has been recently approved, and is now widely used, for the treatment of BPH. Other applications of this drug are now being studied,

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for the treatment of prostatic cancer [5,6] and male pattern baldness [7,8]. To support clinical studies it is therefore important to have a precise, accurate and sensitive method to determine finasteride in biological fluids. The methods developed since now are based on the use of HPLC coupled with an UV detector [9,10] or, recently, with an atmospheric pressure chemical ionization (APCI) tandem mass spectrometric (MS–MS) detector [11]. The sensitivity limit reached with the UV detector is 1 ng/ml and, due to the low wavelength of detection (210 nm), it results scarcely selective and requires an efficient and highly controlled sample preparation with a complex chromatographic system: finasteride is purified from biological samples using a solid-phase extraction (SPE) CN column and analyzed with an HPLC system equipped with two coupled reversed-phase columns maintained at room temperature. The recent use of the mass spectrometric detector [11] has increased both the sensitivity (200 pg/ml) and the selectivity of the assay but, in the method described, the chromatographic system is still complex requiring a series of two reversed-phase columns maintained at 70°C; moreover the MS–MS detector used is quite expensive and the complexity of the apparatus requires an accurate management to perform a quantitative routinely assay.

In this paper we describe a new method based on isotopic dilution gas chromatography–mass spectrometry for the determination of finasteride in human plasma using a GC–MS bench-top instrument. The present method results accurate, specific, sensible and reliable for a routinely determination of finasteride at picogram levels, and was applied to the determination of the pharmacokinetic profile of finasteride in plasma of five male volunteers treated with a 5-mg oral dose of the drug.

2. Experimental

2.1. Materials

Finasteride was a kind gift of Merck Sharp & Dohme (Dr. Elisabetta Stoner). The solvents

employed for the synthesis were analytical grade and were purchased from Carlo Erba (Milan, Italy); all the reagents used for the synthesis were purchased from Sigma (St. Louis, MO, USA). The solvents employed for the purification procedure were analytical grade and were purchased from J.T. Baker (Phillipsburg, NJ, USA); columns used for the purification were SPE C₁₈ (500 mg) *IST* Isolute (Mid Glamorgan, UK); GC capillary column used was an HP1-12 m × 0.2 mm × 0.33 μm obtained from Hewlett-Packard (Palo Alto, CA, USA). All analyses were performed using a Hewlett-Packard GC–MS system composed by a 5890 series II gas chromatograph equipped with a 5971A Mass Spectrometry Detector and a 7673A automatic injector.

2.2. Methods

Synthesis of deuterated internal standard 5,6,6-[²H₃]finasteride (4)

5,6,6-[²H₃]-3-Oxo-4-aza-androstane-17β-carboxylic acid (1)

3-Oxo-4-aza-5-androstene-17β-carboxylic acid (0.81 g, 2.55 mmol), prepared according to the procedure described by Rasmusson et al. [1,12] was stirred in ²H₂O for 16 h at room temperature and, after filtration, in CH₃COO²H (12 ml) for 3 h. Then, the CH₃COO²H solution was maintained for 48 h under ²H₂ (3 atm) at 60°C in the presence of Pt₂O (0.12 g, 0.53 mmol). The solution was then filtered, evaporated and washed with water affording 5,6,6-[²H₃]-3-oxo-4-aza-androstane-17β-carboxylic acid (1) (90% yield, 2.29 mmol).

5,6,6-[²H₃]-3-Oxo-4-aza-androst-1-ene-17β-carboxylic acid (2)

Bis(trimethylsilyl)trifluoroacetamide (BSTFA) (3.8 ml, 14.3 mmol) was added to a stirred suspension of 5,6,6-[²H₃]-3-oxo-4-aza-androstane-17β-carboxylic acid (1) (1.1 g, 3.41 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.79 g, 3.48 mmol) in 11 ml of anhydrous dioxane. The reaction mixture was

stirred at room temperature under N_2 for 4 h and then was refluxed for 18 h. The resulting dark red solution was poured into a mixture containing 20 ml of CH_2Cl_2 and 5.3 ml of a 1% aqueous solution of sodium bisulphite. After vigorous stirring the mixture was filtered to eliminate the precipitated hydroquinone, the organic layer was separated, washed with 7 ml of 2 M HCl and dried over Na_2SO_4 . After removal of the solvent, the residue was washed with CH_3CN and dried under vacuum affording 5,6,6- $[^2H_3]$ -3-oxo-4-aza-androst-1-ene-17 β -carboxylic acid (2) (70% yield, 2.39 mmol).

S-2-Pyridyl-3-oxo-4-aza-androst-1-ene-17 β -thio-carboxylate (3).

A solution of the 5,6,6- $[^2H_3]$ -3-oxo-4-aza-androst-1-ene-17 β -carboxylic acid (2) (0.75 g, 2.34 mmol), with triphenylphosphine (1.03 g, 4.67 mmol) and 2,2'-dithiopyridine in 5 ml of toluene was stirred at room temperature for 6 h, then the solvent was evaporated under vacuum and the residue purified on a short silica gel column (70–230 mesh, 7 cm \times 4 cm I.D.), using acetone–dichloromethane 1:1 (v:v) as eluent, to give *S*-2-pyridyl-3-oxo-4-aza-androst-1-ene-17 β -carboxylate (3) (56% yield, 1.31 mmol).

5,6,6- $[^2H_3]$ -*N*-tert.-butyl-3-oxo-4-aza-5 α -androst-1-ene-17 β -carboxamide (4).

The *S*-2-pyridyl-3-oxo-4-aza-androst-1-ene-17 β -carboxylate (3) (0.25 g, 0.6 mmol) was suspended in 6 ml of anhydrous tetrahydrofuran with 0.5 ml (4.56 mmol) of *tert.*-butylamine; the suspension was stirred at room temperature for 18 h, the solvent was evaporated under vacuum and the residue purified on a short silica gel column (70–230 mesh, 6 cm \times 2.8 cm I.D.) using a mixture of acetone–dichloromethane 1:3 (v:v) as eluent, obtaining the 5,6,6- $[^2H_3]$ -*N*-tert.-butyl-3-oxo-4-aza-5 α -androst-1-ene-17 β -carboxamide ($[^2H_3]$ finasteride) (4) (43% yield, 0.26 mmol). The percentage of deuteration was obtained from selected ion monitoring (SIM) analysis focusing on the ions at $m/z = 375.2$ ($^2H_3 = 64.2\%$), $m/z = 374.2$ ($^2H_2 = 30.96\%$), $m/z =$

373.2 ($^2H_1 = 4.57\%$), $m/z = 372.2$ ($^2H_0 = 0.45\%$).

Instrumental conditions

Analyses were performed using a 12 m \times 0.2 mm \times 0.33 μ m GC capillary column with a 100% methylsilicone phase. The temperature program was: 70°C \times 1 min; then 30°C/min to 200°C; 200°C \times 1 min; then 10°C/min to 280°C; 280°C \times 10 min; then 20°C/min to 300°C for 5 min. The transfer line temperature was 280°C. The carrier gas was helium with an inlet pressure of 35 KPa. In these conditions the retention time of finasteride and its trideuterated analogue was 19.7 min. Injections were performed in the splitless mode with a purge-off time of 1 min and an injector temperature of 280°C. SIM analyses were made acquiring the molecular ions at m/z 372.2 and 375.2 for finasteride and $[^2H_3]$ finasteride respectively.

Analytical method

Extraction and purification of finasteride and I.S. from plasma samples were performed using SPE C_{18} columns (500 mg). Columns were pre-washed with 2 \times 3 ml of ethyl acetate and 3 ml of methanol, then conditioned with 3 ml of methanol and 3 ml of water. Plasma samples were diluted 1:5 with water and applied to columns, then columns were washed with 2 \times 2.5 ml of bidistilled water, 3 ml of *n*-hexane and successively with 3 \times 3 ml of *n*-hexane–ethyl ether 60:40 (this fraction contained testosterone and dihydrotestosterone). Finasteride was then eluted with 3 \times 3 ml of ethyl acetate, the eluate was dried on a short column of anhydrous sodium sulphate and then evaporated to dryness under nitrogen. Dry residues were dissolved in 15 μ l of *n*-heptane and 3 μ l auto injected into the GG–MS instrument for the quantitative determination. The amount of finasteride in plasma was determined by SIM analyses acquiring the molecular ions at m/z 372.2 for finasteride and m/z 375.2 and $[^2H_3]$ finasteride (I.S.). For each sample the peak-area ratio (PAR, area of finasteride peak versus area of $[^2H_3]$ finasteride peak) was

measured and the amount of finasteride determined from the calibration curve.

Selectivity and linearity of the method

Selectivity of the method was evaluated by analyzing blank samples and finasteride-free plasma samples. The calibration curve was made with eight points in the range 0–30 ng/ml of plasma and was obtained by adding the appropriate amounts of finasteride and of the deuterated standard (5 ng) to plasma aliquots of 1 ml. Each point of the calibration curve was purified and analyzed as described and PAR were plotted versus finasteride concentration. A good linearity was obtained from 0 to 30 ng/ml ($y = 0.214x - 0.045$; $r = 0.997$)

Recovery

Analytical recoveries were calculated as described by Dehennin [13]: identical samples of 1 ml of plasma containing finasteride at the concentration of 5 ng/ml were supplemented with 5 ng of the deuterated internal standard (I.S.), either before or after the extraction and purification procedure. For each sample the peak-area ratio (PAR, area of finasteride peak versus area of [$^2\text{H}_3$]finasteride peak) was calculated and percentage of recovery was calculated as $\text{PAR (I.S. added before purification) / PAR (I.S. added after purification)} \times 100$.

Sensitivity

Sensitivity limit of the instrument was evaluated by injection of decreasing amounts of finasteride and calculating the signal-to-noise ratio (S/N); 50 pg of finasteride injected gave a $S/N = 6.4$ (Fig. 1a). Sensitivity limit of the assay was evaluated analysing plasma samples (1 ml) at decreasing concentration of finasteride and calculating the S/N ; samples at 250 pg/ml gave a $S/N = 6.2$ when 3 μl of the 15 μl final volume were injected (Fig. 1b).

Precision and accuracy of the method

The precision and accuracy of the method were determined by replicate inter-day analyses ($n = 5$) of plasma samples (1 ml) at the following finasteride concentrations: 1, 5, 8 and 10 ng/ml.

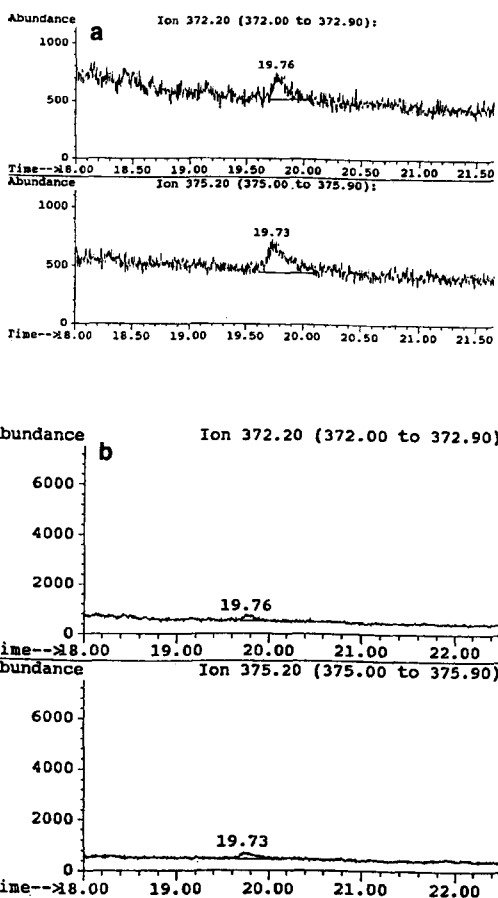


Fig. 1. (a) Plots from computer of the signal corresponding to the injection of 50 pg of finasteride ($m/z = 372.2$) and 50 pg of $^2\text{H}_3$ -finasteride ($m/z = 375.2$). The S/N ratio is 6.4. (b) Plots from computer of the signal corresponding to a 1-ml plasma sample of 250 pg/ml of finasteride ($m/z = 372.2$) and 250 pg/ml of $^2\text{H}_3$ -finasteride ($m/z = 375.2$) processed according to the analytical method and injected in the GC-MS system (3- μl injection of the 15- μl final volume of heptane). S/N ratio is 6.2.

Precision was evaluated by the percent coefficient of variation (CV.%) and accuracy was calculated as mean calculated concentration versus nominal concentration (Table 1).

Pharmacokinetic profile

The pharmacokinetic profile was evaluated on five healthy male volunteers aged from 27 to 58 and subjected to a single oral 5-mg dose of finasteride. Blood samples were collected before (time point 0) and 1, 2, 4, 8 and 24 h after oral

Table 1
Precision and accuracy of the method^a

Nominal concentration (ng/ml)	Concentration found (mean ± S.D., <i>n</i> = 5) (ng/ml)	Accuracy (%)	Precision (C.V., %)
1.00	1.10 ± 0.14	110	12.4
5.00	5.34 ± 0.31	106	5.81
8.00	8.17 ± 0.22	102	2.70
10.00	9.95 ± 0.40	99	4.00

^a Precision and accuracy of the method were determined by replicate inter-day analyses (*n* = 5) of plasma samples (1 ml). Accuracy is calculated as: (mean calculated concentration/nominal concentration) × 100.

administration. Immediately after collection the samples were centrifuged and plasma was stored at -20°C until analysis. Plasma aliquots (from 0.3 to 1 ml) were diluted 1:5 (v/v) with bidistilled water and the internal standard (5 ng, in 50 μl of ethanol) was added. Samples were equilibrated at 37°C for 1 h before analysis and then processed according to the above-described analytical method.

The kinetic parameters were calculated as follow [14]: C_{\max} was the maximum concentration of the pharmacokinetic profile and t_{\max} was the time at which the maximum concentration was reached. The $t_{1/2}$ was calculated as:

$$t_{1/2} = \frac{0.693}{K_{el}}$$

where K_{el} is the elimination constant. The AUC_{0-24} was calculated with the trapezoidal rule.

3. Results and discussion

3.1. Synthesis of the deuterated internal standard

5,6,6-²H₃]Finasteride (4) was prepared from 3-oxo-4-aza-5-androstene-17β-carboxylic acid in a four-step procedure, as shown in Fig. 2. In the first step three deuterium atoms were introduced: first at position 6 by isotopic exchange in ²H₂O and CH₃COO²H and then at position 5 and 6 by reduction with ²H₂ of the 5–6 double bound. The

IR, ¹H-NMR, ¹³C-NMR and MS spectra of deuterated compound 1 were identical with the spectra reported for the hydrogenated compound [1,12]. The molecular ion in the MS spectrum at 322 *m/z*, the absence in the ¹³C-NMR spectrum of the C5 and C6 signals at 60.03 and 26.54 ppm, respectively, and the lack in the ¹H-NMR spectrum of the signal at 2.95 ppm corresponding to the H-C5 hydrogen are consistent with the introduction of three deuterium atoms at the positions 5,6,6. The conversion of the trideuterated compound (1) to ²H₃-finasteride was made according to the reported procedure for the synthesis of finasteride. The procedure requires the introduction of a double bond in position 1–2, followed by activation of the carboxylic group and reaction with *tert.*-butylamine. The percentages of deuterated compounds obtained from selected ion monitoring (SIM) analysis were: [²H₃]finasteride 64.2%; [²H₂]finasteride 30.96%; [²H₁]finasteride 4.57% and non-deuterated finasteride 0.45%. The high ratio of the trideuterated compound versus the non-deuterated one (²H₀/²H₃ = 0.007) allows an optimal use of [²H₃]finasteride as internal standard in an isotope dilution method.

3.2. Analytical method

This new method for the determination of finasteride in human plasma is the first one that uses a gas chromatography–mass spectrometry technique and it resulted very sensitive, accurate and precise owing to the use of a deuterated internal standard.

The analytical procedure is simple and it can be easily automated for analysis of a large number of samples. The extraction and purification of finasteride from plasma was performed using only a single-step procedure on a SPE-C₁₈ column. The choice of the washing solvents allowed the separation of T and DHT from finasteride which was eluted with ethyl acetate. After removal of ethyl acetate, a heptane solution of the sample was injected directly into the GC–MS instrument without derivatization. The sensitivity of the method was at picogram level (250 pg/ml of plasma), analysing 1 ml of plasma

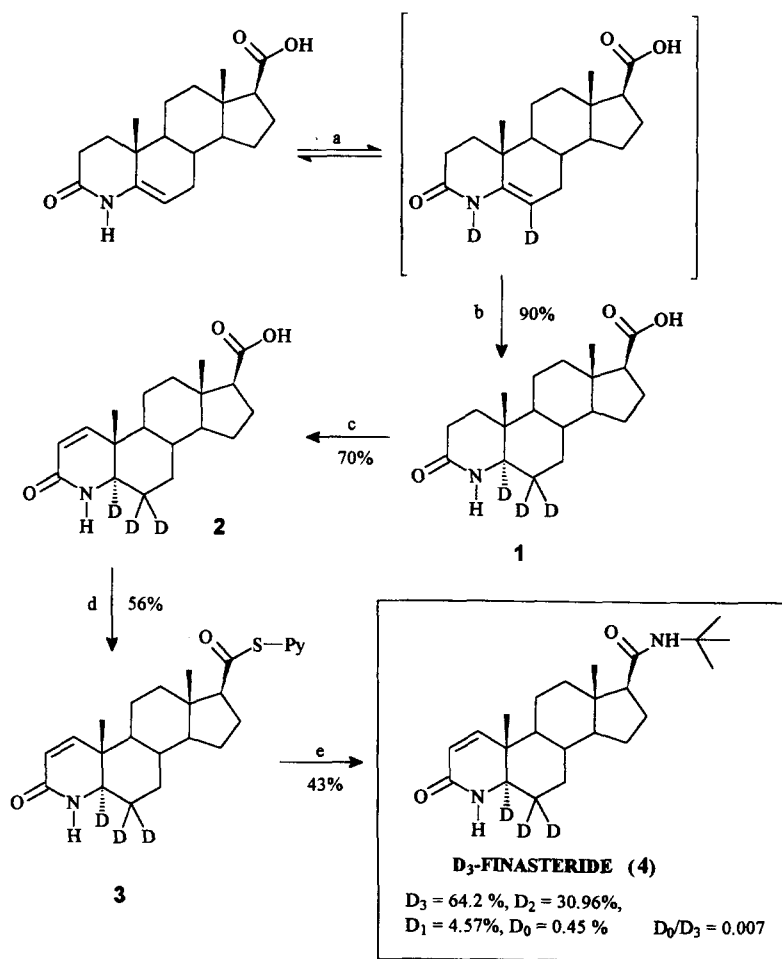


Fig. 2. Synthesis of [²H₃]finasteride. (a) ²H₂O 16 h, CH₃COO²H 3 h; (b) ²H₂, PtO₂, CH₃COO²H, 3 atm, 60°C, 48 h; (c) BSTFA, DDQ, dioxane, 25°C, 4 h, then 101°C, 18 h; (d) 2,2'-dithiopyridine, PPh₃, toluene, 25°C, 6 h; (e) *tert.*-butylamine, THF, 25°C, 18 h. [²H₃] is the trideuterated compound, [²H₂] the dideuterated compound, [²H₁] the monodeuterated compound and [²H₀] the non-deuterated compound.

and injecting 3 μ l of the sample dissolved in 15 μ l of heptane. The sensitivity of the method was increased to 80 pg/ml of plasma when 3 ml of plasma were used without any change in the isolation and purification procedure. The same sensitivity was also obtained by dissolving the dried eluate in 5 μ l of heptane and manually injecting 3 μ l of solution. However, the methodology giving the sensitivity of 250 pg/ml was sufficient for the analysis of plasma samples of the volunteers. Mean recovery for finasteride was

90% ($n = 6$). No interferences were found deriving from materials used or from endogenous compounds of plasma. Accuracy and precision shown in Table 1 attest a good reproducibility of the analyses.

This method has been applied to the determination of the plasmatic pharmacokinetic profile of finasteride in five male volunteers treated with a single oral 5-mg dose of drug. The pharmacokinetic profiles (illustrated in Fig. 3) and the kinetic parameters calculated (reported in Table

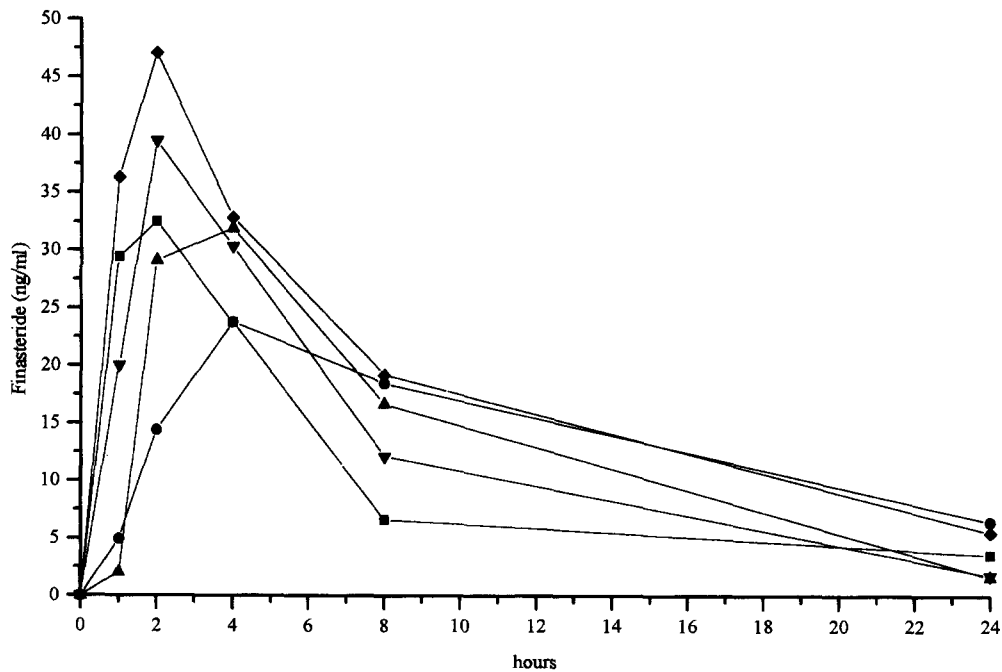


Fig. 3. Pharmacokinetic profiles of finasteride in plasma of five healthy male volunteers treated with a single 5-mg oral dose of the drug.

2) are in excellent agreement with the pharmacokinetic profile and the kinetic values obtained by other authors for the same dose of drug [10].

The purification procedure of the samples

allows the separation of T and DHT from finasteride and could be applied to the simultaneous determination of the drug and the two hormones by GC–MS. Some experiments are in progress in order to apply this methodology to clinical

Table 2
Pharmacokinetic parameters of finasteride in plasma^a

	C_{\max} (ng/ml)	T_{\max} (h)	$t_{1/2}$ (h)	AUC_{0-24} (ng h/ml)
Volunteer 1	32.5	2	6.9	243.8
Volunteer 2	23.7	4	11.5	333.2
Volunteer 3	31.9	4	4.9	320.5
Volunteer 4	39.4	2	4.9	305.0
Volunteer 5	47.1	2	7.7	441.0
GC–MS method mean \pm S.D. ($n = 5$)	34.9 ± 8.8	2.8 ± 1.1	7.2 ± 2.7	328.5 ± 71.6
Reported values ^b	33.6 ± 8.3	1.5	6.9	312.45

^a Values calculated from the pharmacokinetic profile of five male healthy volunteers treated with a single oral 5-mg dose of finasteride.

^b These values were calculated from the pharmacokinetic profile described by Costanzer et al. [10].

studies on patients affected by BPH under treatment with finasteride.

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References

- [1] G.H. Rasmusson, G.F. Reynolds, N.G. Steinberg, E. Walton, G.F. Patel, T. Liang, M.A. Cascieri, A.H. Cheung, J.R. Brooks and C. Berman, *J. Med. Chem.*, 29 (1986) 2298.
- [2] H.O. Beisland, B. Binkowitz, E. Brekkan, P. Ekman and M. Konturri, *Eur. Urol.*, 22 (1992) 271.
- [3] E. Stoner, Finasteride Study Group, *J. Urol.*, 147 (1992) 1298.
- [4] G.J. Gormley, E. Stoner, R.C. Bruskewitz, J. Imperato-McGinley, P.C. Walsh, J.D. McConnel, G.L. Andriole, J. Geller, B.R. Bracken, J.S. Tenover, E. Darracott Vaughan, F. Pappas, A. Taylor, B. Brinkowitz and J. Ng, *New Engl. J. Med.*, 327 (1992) 1185.
- [5] O.W. Brawley, G.L. Ford, I. Thompson, J.A. Perlman and B.S. Kramer, *Cancer Epidemiol. Biomarkers Prev.*, 3 (1994) 177.
- [6] L.G. Ford, O.W. Brawley, J.A. Perlman, S.G. Nayfield, K.A. Johnson and B.S. Kramer, *Cancer*, 74 (1994) 2726.
- [7] A.L. Dallob, N.S. Sadik, W. Unger, S. Lipert, L.A. Geissler, S.L. Gregoire, H.H. Nguyen, E.L. Moore and W.K. Tanaka, *J. Clin. Endocrinol. Metab.*, 79 (1994) 703.
- [8] L. Rhodes, J. Harper, H. Uno, G. Gaito, J. Audette-Arruda, S. Kurata, C. Berman, R. Primka and B. Pikounis, *J. Clin. Endocrinol. Metab.*, 79 (1994) 991.
- [9] J.R. Carlin, P. Christofalo and W.J.A. Vandenheuvel, *J. Chromatogr.*, 427 (1988) 79.
- [10] M.L. Costanzer, B.K. Matuszewski and W.F. Bayne, *J. Chromatogr.*, 566 (1991) 127.
- [11] M.L. Costanzer, C.M. Chavez and B.K. Matuszewski, *J. Chromatogr.*, 658 (1994) 281.
- [12] G.H. Rasmusson, G.F. Reynolds, T. Utne, R.B. Jobson, R.L. Primka, C. Berman and J.R. Brooks, *J. Med. Chem.*, 27 (1984) 1690.
- [13] L. Dehennin, *Clin. Chem.*, 35 (1989) 532.
- [14] J.R. Carlin, P. Höglund, L.-O. Eriksson, P. Christofalo, S.L. Gregoire, A.M. Taylor and K.E. Andersson, *Drug Metab. Dispos.*, 20 (1992) 148.