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# Determination of finasteride in human plasma by liquid–liquid extraction and high-performance liquid chromatography

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**Abstract**

A high-performance liquid chromatographic method for the quantitation of finasteride in human plasma is presented. The method is based on liquid–liquid extraction with hexane–isoamylalcohol (98:2, v/v) and reversed-phase chromatography with spectrophotometric detection at 210 nm. The mobile phase consists of acetonitrile–15 m*M* potassium dihydrogenphosphate (40:60, v/v). Clobazam is used as the internal standard. The limit of quantitation is 4 ng/ml and the calibration curve is linear up to 300 ng/ml. Within-day and between-day precision expressed by relative standard deviation is less than 5% and inaccuracy does not exceed 8%. The assay was used for pharmacokinetic studies. 

*Keywords:* Finasteride

## 1. Introduction

Finasteride (Fig. 1) is a synthetic 4-azasteroid compound used for the treatment of benign prostatic hyperplasia. The mechanism of action is through specific inhibition of steroid 5α-reductase which converts testosterone to the potent androgen 5α-dihydrotestosterone responsible for the enlargement of the prostate gland [1].

Several HPLC methods have been reported for the determination of finasteride in biological fluids. Although combination with tandem mass spectrometry was used in one report [2], most of published methods employ spectrophotometric detection.

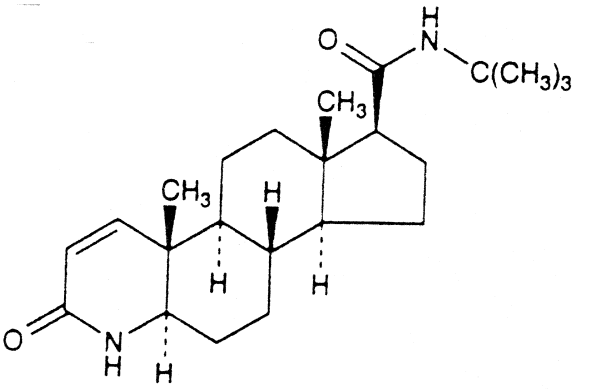


Fig. 1. Structure of finasteride.

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As detection at 210 nm must be used, intensive sample clean up before analysis is necessary. The sample pre-treatment is based exclusively on column switching [3] or solid-phase extraction (SPE) [2,4–6]. Unfortunately a two-stage cleaning process with different cartridges seems to be necessary to obtain clean chromatograms. The limit of quantitation (LOQ) of published procedures varies from 1 to 10 ng/ml, which reflects the complexity and length of the sample pre-treatment. Most of published methods use a commercially unavailable methylderivative of finasteride as an internal standard.

In addition, one gas chromatographic–mass spectrometric method with a SPE purification step and a LOQ at picogram level was published [7].

We present a simple method based on a one-step liquid–liquid extraction with a commercially available internal standard – clobazam. The sensitivity of the method is sufficient for pharmacokinetic studies.

## 2. Experimental

### 2.1. Chemicals

Finasteride (as a free base) was obtained from Cipla Ltd. (Mumbai Central, Mumbai, India), acetonitrile (Chromasolv, for LC) was from Riedel de Haën, hexane (spectroscopic-grade, Uvasol) and potassium dihydrogenphosphate (analytical-grade) were from Merck (Darmstadt, Germany), isoamylalcohol was from Fluka Chemie AG (Buchs, Switzerland) and clobazam (internal standard) was obtained in a local pharmacy in the form of tablets (10 mg tablets, Frisium 10, Hoechst AG, Germany).

### 2.2. Apparatus

All HPLC instruments were obtained from Thermo Separation Products (Riviera Beach, FL, USA). The system consisted of the membrane degasser, pump ConstaMetric 4100, automatic sample injector AS 3000, spectrophotometric detector UV2000 and datastation with PC1000 software, version 2.5. The separation was performed on Nucleosil 100-3 $C_{18}$ particle size 3 μm, 150×3.2 mm I.D. column (Watrex, Prague, Czech Republic). A pre-column 10×4 mm I.D. packed with Nucleosil 120-5 $C_{18}$, particle size 5 μm, was used.

The mobile phase consisted of acetonitrile–15 m*M* potassium dihydrogenphosphate buffer (40:60, v/v). The flow-rate of the mobile phase was 0.6 ml/min at 30°C. The spectrophotometric detector was operated at 210 nm with the time constant 2 s.

### 2.3. Standards

Stock solutions of finasteride were made by dissolving approximately 10 mg in 10 ml of methanol. Separate solutions were prepared for calibration curve and quality control samples. Further solutions were obtained by serial dilutions of the stock solutions with methanol. These solutions were added to drug-free plasma in volumes not exceeding 1% of the plasma volume.

One Frisium 10 tablet was disintegrated in 10 ml of methanol in the ultrasonic bath. The mixture was centrifuged at 2600 *g* for 10 min, the supernatant was diluted to 1:49 with methanol and used as the internal standard. The solution was stable for 1 month, all samples and standards were analyzed with the same solution of internal standard.

All solutions were stored at −18°C and protected from light.

### 2.4. Preparation of the sample

The samples were stored in the freezer at −18°C and allowed to thaw at room temperature before processing.

A 10-μl aliquot of the internal standard solution (200 ng of clobazam) was added to 1 ml of plasma and the tube was briefly shaken. Then the mixture was vortex mixed with 4 ml of hexane–isoamylalcohol (98:2, v/v) for 2 min at 1800 rpm. The tube was centrifuged 5 min at 2600 *g*, the upper organic phase was transferred to another tube and evaporated to dryness under the stream of nitrogen at 60°C. The residue was dissolved in 100 μl of the mixture acetonitrile–15 m*M* potassium dihydrogenphosphate buffer (1:1 v/v). The sample was transferred to the polypropylene autosampler vial and 30 μl were injected into the chromatographic system.

### 2.5. Calibration curves

The calibration curve was constructed in the range 4–300 ng/ml to encompass the expected concentrations in measured samples. The calibration curves were obtained by weighted linear regression (weighing factor: $1/y^2$): the ratio of finasteride peak height to clobazam peak height was plotted vs. the ratio of finasteride concentration to that of the internal standard in ng/ml. The suitability of the calibration model was confirmed by back-calculating the concentrations of the calibration standards.

### 2.6. Limit of quantitation

Limit of quantitation (LOQ) was defined as the lowest concentration at which the precision expressed by relative standard deviation (RSD) is better than 20% and accuracy expressed by relative difference of the measured and true value is also lower than 20%. Six identical samples were analyzed for the determination of LOQ.

## 3. Results and discussion

### 3.1. Chromatography

Finasteride produces a symmetric sharp peak when chromatographed under described conditions. Its retention behaviour is not dependent on the pH of the mobile phase in the range 3–6.5; thus, 15 m*M* phosphate buffer without additional pH adjustment was used. Also the column temperature did not affected the peak shape, so the column was held at 30°C.

The retention time of clobazam and finasteride was 10 and 17 min, respectively, under the described chromatographic conditions. The total run time was set to 25 min to overcome problems with late eluting peaks. The column efficiency, expressed by the number of theoretical plates, was better than 5000 and peak asymmetry measured at 5% of the peak height was better than 1.2 for both compounds.

The method selectivity was demonstrated on six blank plasma samples obtained from healthy volunteers: the chromatograms were found to be free of interfering peaks. The typical chromatogram of blank plasma is shown in Fig. 2. The chromatogram of a plasma sample 11 h after administration of 10 mg of finasteride to a volunteer is shown in Fig. 3. The concentration of finasteride was 19.7 ng/ml.

### 3.2. Sample preparation

Thorough removal of interfering compounds is necessary due to the non-selective spectrophotometric detection at 210 nm. Initial attempts with SPE methods resulted in the conclusion that consecutive combination of two cartridges with different sorbents would be necessary to obtain a clean sample. Moreover, larger volumes of organic solvents, approximately 5 ml, compared with the usually reported volumes of 1–2 ml, were necessary to elute interfering compounds from the cartridges. Thus the main advantage of the SPE method – speed – is lost and, moreover, problems with waste solvent handling

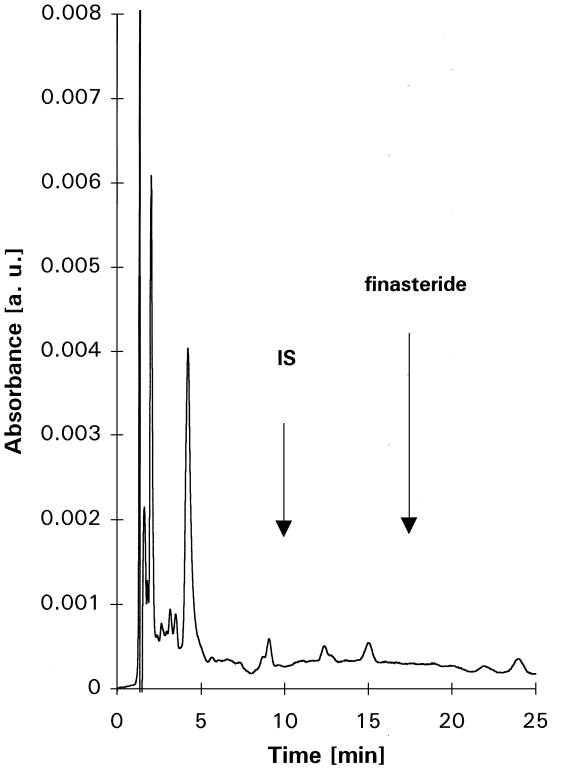


Fig. 2. Typical chromatogram of drug-free human plasma. The arrows indicate the retention time of finasteride and clobazam (internal standard).

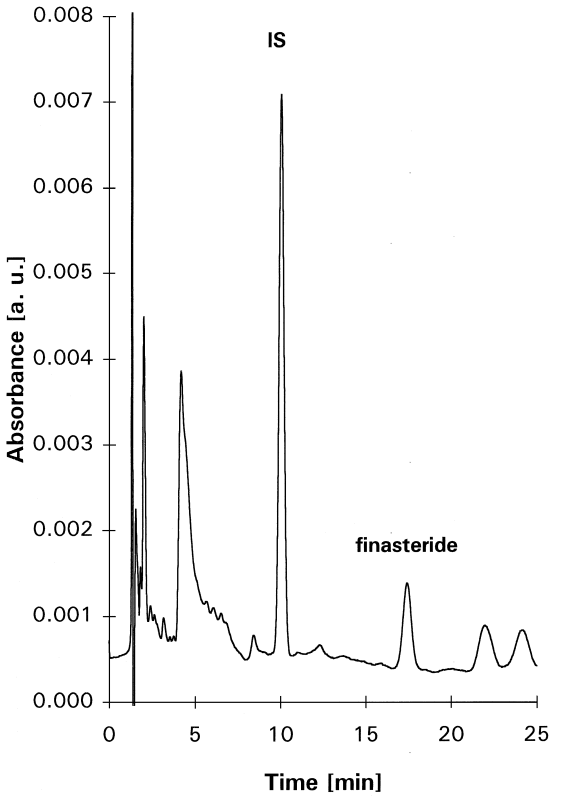


Fig. 3. Chromatogram of a plasma sample from a volunteer 11 h after administration of 10 mg of finasteride. The respective concentration was 19.7 ng/ml.

arose. Our attention was therefore focused on simple and more cost-effective liquid–liquid extraction. Pure hexane produced low (40–50%) finasteride recoveries, but addition of 2% of isoamylalcohol resulted in 90% and 65% recovery of finasteride and the internal standard, respectively. Dichloromethane extraction of finasteride is also very efficient (nearly 100%), but more interfering compounds are extracted. Although finasteride contains a secondary amine in its side chain, it does not undergo back-extraction to the acid, probably due to the large lipophilic part of the molecule.

### *3.3. Linearity and limit of quantitation*

The calibration curves were linear in the studied range. The mean equation (curve coefficients±SD) of the calibration curve ($n=9$) obtained from six points was $y = 0.740(\pm 0.055)x - 0.0047(\pm 0.0118)$ (correlation coefficient $r=0.9991$), where $y$ represents the finasteride peak height to clobazam peak height ratio and $x$ represents the ratio of finasteride concentration to that of the internal standard.

The LOQ was 4.08 ng/ml. The precision, characterised by the RSD, was 8.4% and accuracy, defined as the deviation between the true and the measured value expressed in percents, was 2.4% at this concentration ($n=6$).

Table 1
Intra-day precision and accuracy

| $n$[a] | Concentration (ng/ml) | | Bias (%) | RSD (%) |
|---|---|---|---|---|
| | Added | Measured | | |
| 6 | 8.169 | 7.851 | −3.9 | 3.4 |
| 6 | 36.66 | 36.15 | −1.4 | 0.8 |
| 6 | 280.3 | 270.0 | −3.7 | 0.7 |

[a] $n$ = Number of samples.

#### *3.3.1. Intra-day precision*

Intra-day precision of the method is illustrated in Table 1. Six sets of quality control samples (low, medium and high concentrations) were analysed with calibration samples in day. Both precision and accuracy were better than 4% at all levels.

#### *3.3.2. Inter-day precision and accuracy*

Inter-day precision and accuracy was evaluated by processing a set of calibration and quality control samples (three levels analysed twice and the results were averaged for statistical evaluation) on six separate days. The samples were prepared in advance and stored at −18°C. The respective data are given in Table 2. The precision was better than 8% and the inaccuracy did not exceed 5% at all levels.

Table 2
Inter-day precision and accuracy

| $n$[a] | Concentration (ng/ml) | | Bias (%) | RSD (%) |
|---|---|---|---|---|
| | Added | Measured | | |
| 6 | 8.169 | 8.158 | −0.1 | 7.1 |
| 6 | 36.66 | 35.56 | −3.0 | 3.2 |
| 6 | 280.3 | 268.3 | −4.3 | 1.6 |

[a] $n$ = number of days.

### 3.3.3. Stability study

*3.3.3.1. Freeze and thaw stability*. An aliquot of 10 ml of a low and high concentration sample were prepared. The solutions were stored at −18°C and subjected to three thaw and freeze cycles. During each cycle duplicate 1 ml aliquots were processed, analysed and the results averaged. The results are shown in Table 3. The concentrations found are within the allowed limit ±15% of nominal concentration, indicating no significant substance loss during repeated thawing and freezing.

*3.3.3.2. Processed sample stability*. Two sets of samples (8.169 ng/ml as a low and 280.3 ng/ml as a high concentration of finasteride) were analysed in one day and left in the autosampler at ambient temperature. The samples were analysed using a freshly prepared calibration samples 2 days later. The results are presented in Table 4. The processed samples are stable at room temperature for 2 days.

*3.3.3.3. Long term stability*. Two sets of samples (low and high concentration of finasteride) were stored in the freezer at −18°C for 6 weeks. The samples were then analysed using freshly prepared calibration samples. The results are presented in Table 5. The samples are stable at −18°C for at least 6 weeks.

Table 5
Long-term stability of the samples

| Concentration (ng/ml) | $n$[a] | Concentration found (ng/ml) | RSD (%) | Bias (%) |
|---|---|---|---|---|
| 16.33 | 6 | 14.28 | 0.9 | −12.6 |
| 280.3 | 6 | 265.1 | 2.9 | −5.4 |

[a] $n$ = number of samples.

### 3.4. Application to biological samples

The proposed method was applied to the determination of finasteride in plasma samples for the purpose of the bioequivalence study. Plasma samples were periodically collected up to 34 h after oral administration of two 5-mg tablets to 26 healthy male volunteers. Fig. 4 shows the mean plasma concentration of finasteride. The plasma level of finasteride reached a maximum 2 h after the administration and thereafter the plasma level declined with an elimination half-time of ca. 6 h. These values agree with previously published reports [1]. The extrapolated fraction of the AUC from 0 to infinity accounted only for 10%, which indicates a suitability of the analytical method for pharmacokinetic studies.

Table 3
Freeze and thaw stability of the samples

| Sample concentration (ng/ml) | $n$[a] | Cycle 1 | | Cycle 2 | | Cycle 3 | |
|---|---|---|---|---|---|---|---|
| | | Measured | Bias (%) | Measured | Bias (%) | Measured | Bias (%) |
| 16.33 | 3 | 15.69 | −3.9 | 15.64 | −4.2 | 16.40 | −0.4 |
| 280.3 | 3 | 270.6 | −3.5 | 254.8 | −9.1 | 272.2 | −2.9 |

[a] $n$ = number of samples.

Table 4
Processed sample stability of the samples

| Sample | Concentration (ng/ml) | $n$[a] | Concentration found (ng/ml) | RSD (%) | Bias (%) |
|---|---|---|---|---|---|
| New | 7.858 | 6 | 7.121 | 11.7 | −9.4 |
| 2-Days-old | 7.858 | 5 | 8.235 | 6.0 | 4.8 |
| New | 280.3 | 6 | 265.1 | 2.9 | −5.4 |
| 2-Days-old | 280.3 | 6 | 274.7 | 4.0 | −2.0 |

[a] $n$ = number of samples.

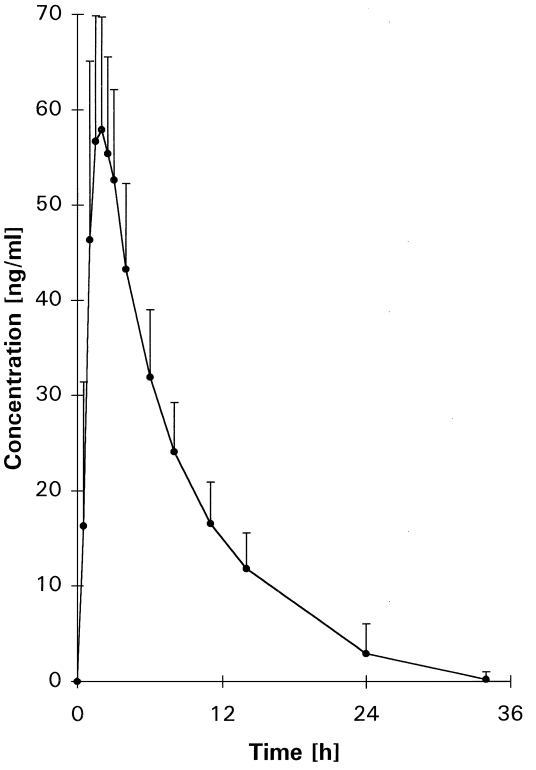


Fig. 4. Mean plasma concentrations (+SD) of finasteride after a 10 mg single oral dose (26 healthy volunteers).

## 4. Conclusions

The validated method allows determination of finasteride in the 4–300 ng/ml range. The sample preparation is simple, rapid and cheap. The precision and accuracy of the method is fully comparable with the previously published procedures and the sensitivity of the assay is sufficient to follow the pharmacokinetics of this drug.

## References

[1] D.H. Peters, E.M. Sorkin, Drugs 46 (1993) 177.
[2] M.L. Constanzer, C.M. Chavez, B.K. Matuszewski, J. Chromatogr. B 658 (1994) 281.
[3] T. Takano, S. Hata, J. Chromatogr. B 676 (1996) 141.
[4] J.R. Carlin, P. Christofalo, W.J.A. Vandenheuvel, J. Chromatogr. 427 (1988) 79.
[5] M.L. Constanzer, B.K. Matuszewski, W.F. Bayne, J. Chromatogr. 566 (1991) 127.
[6] G. Carlucci, P. Mazzeo, J. Chromatogr. B 693 (1997) 245.
[7] A. Guarna, G. Danza, G. Bartolucci, A. Marrucci, S. Dini, M. Serio, J. Chromatogr. B 674 (1995) 197.