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Short communication

# A rapid, simple, specific liquid chromatographic–electrospray mass spectrometry method for the determination of finasteride in human plasma and its application to pharmacokinetic study

Fang-Qiu Guo [a,b], Lan-Fang Huang [a,c,*], Kin Ping Wong [b], Yun-Hui Dai [a,c], Ya-Wen Li [a], Yi-Zeng Liang [a], Ke-Long Huang [a], Ke-Jun Zhong [c], Ming-Jian Wu [c]

[a] College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, PR China
[b] Biology Department of College of Science and Mathematics, California State University-Fresno, Fresno, CA 93740, United States
[c] Technical Center of Changde Cigarette Factory, Changde 415000, PR China

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**Abstract**

A fast, accurate, sensitive, selective and reliable method using reversed-phase high-performance liquid chromatography–mass spectrometry coupling with an electrospray ionization interface was developed and validated for the determination of finasteride in human plasma. After deprotienation with acetonitrile, centrifugation, evaporation to dryness and dissolving in mobile phase, satisfactory separation was achieved on a Hypersil-Keystone $C_{18}$ reversed-phase column using a mobile phase consisting of acetonitrile–water (46:54, v/v), 0.1% acetic acid and 0.1% trifluoracetic acid. Carbamazepine (IS) was used as internal standard. This method involved the use of the $[M+H]^+$ ions of finasteride and IS at *m/z* 373 and 237 with the selective ion monitoring (SIM) mode. The calibration curve was linear in the range of 0.2–120 ng ml$^{-1}$. The limit of quantification for finasteride in plasma was 0.2 ng ml$^{-1}$ with good accuracy and precision. The intra-assay precision and accuracy were in the range of 2.1–11.2% and −1.3% to 8.5%, respectively. The inter-assay precision and accuracy were in the order of 3.4–12.1% and −1.5% to 11.5%, respectively. The mean sample extract recoveries of the method were higher than 85% and 74% for finasteride and internal standard (IS), respectively. The assay has been successfully used to estimate the pharmacokinetics of finasteride after oral administration of a 5 mg tablet of finasteride to 24 healthy volunteers.


*Keywords:* Finasteride; Liquid chromatography–mass spectrometry (LC–MS); Electrospray ionization (ESI); Pharmacokinetic

## 1. Introduction

Finasteride [3-oxo-4-aza-5α-androst-1-ene-17β-(*N-tert*-butylcarboxamide)] (Fig. 1), is a member of the family of compounds referred to as 4-azasteroids [1,2]. It appears to be the most effective and widely used drug for the treatment of benign prostate hyperplasia and is also believed to be the first selected therapeutic drug for the treatment of benign prostate hyperplasia besides operation therapy [3–8]. The functioning mechanism is through specific inhibition of steroid 5a-reductase, which blocks the intracellular metabolism of testosterone. Thereby it inhibits testosterone to be converted into the androgen dihydrotestosterone (DHT), which is responsible for the enlargement of the prostate gland [9,10]. For these reasons, the quantification of finasteride in human plasma is very important in clinical study.

Several methods for the determination of finasteride in biological samples have been developed. These methods include high-performance liquid chromatography (HPLC) [11–14], polarography [15], liquid chromatography–tandem mass spectrometry [16–18] and an isotop dilution mass-spectrometric method [19]. Macek has reviewed about these published papers [20]. HPLC is the often used one. However, HPLC methods mentioned above suffer from limitations such as low sensitiv-

* Corresponding author at: College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, PR China. Tel.: +86 731 8836376; fax: +86 731 8879616.
*E-mail address:* lf18huang@yahoo.com.cn (L.-F. Huang).

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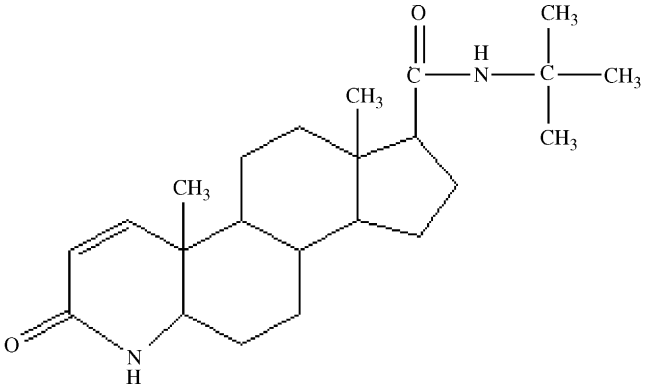
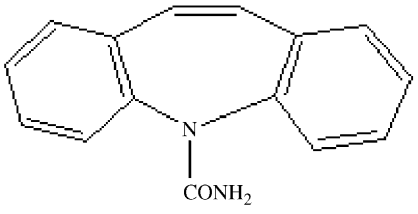
ity, poor selectivity and time-consuming due to complex sample preparation. Among these methods, LC–MS/MS method may have the highest sensitivity, but the determination process is also complex and a two-stage cleaning process with different cartridges seems to be necessary to obtain good selectivity and accuracy. From the reported procedures, the complexity and length of the sample pre-treatment can be reflected by the different limits of quantitation (LOQ), which vary from 0.2 to 10 ng ml$^{-1}$.

LC–MS has become the widely used analytical tool for the pharmacokinetic study and quantification of drugs and metabolites in biological samples due to its high sensitivity and selectivity. Here, for the first time, a fast, sensitive, selective, rapid and accurate liquid chromatographic–electrospray mass spectrometry (LC/ESI-MS) method for the determination of finasteride in human plasma was described. The method was validated with selectivity and matrix effect, linearity range, precision, accuracy, and limit of quantification (LOQ). Using carbamazepine (shown in Fig. 1) as internal standard (IS), after deprotienation with acetonitrile, a LC–single quadrupole mass spectrometer with electrospray ionization source (ESI) was used for the quantification of finasteride using 0.2 ml plasma sample. By analyzing plasma samples from selected subject participating in pharmacokinetic study, the utility of the methodology was demonstrated. After oral administration of 5 mg dose, the concentration versus time profile of finasteride was given in present investigation. The results showed that the developed method was reliable and adequate to provide pharmacokinetic concentration–time profile dosed as low as 5 mg of finasteride.




Finasteride




Carbamazepine

Fig. 1. Chemical structures of finasteride and carbamazepine.

## 2. Experimental

### 2.1. Reagents and chemicals

Finasteride standard and oral administration drugs (5 mg table$^{-1}$), both of which were produced by Hanzhou Xianju Pharmaceutical Co. Ltd. (Hanzhou, PR China), were purchased from China Supervise Institute of Drug and Biological Preparation (Beijing, PR China). Carbamazepine (5*H*-dibenz [*b*, *f*] azepine-5-carboxamide) was purchased from Sigma (St. Louis, MO, USA). Trifluoracetic acid (TFA) was purchased from Merch (Darmstadt, Germany). HPLC-grade acetonitrile and methanol were obtained from Hanbang Science and Technology Co. (Jiangsu, PR China). Acetic acid from Shanghai Chemical Reagents Reagent Plant Ltd. (Shanghai, PR China) was of analytical grade. All solvents were filtered through a 0.45 μm membrane and degassed. All water used was Milli-Q grade (Millipore, Bedford, MA, USA). Stock solutions of finasteride and internal standard (IS) carbamazepine at concentration of 0.1 mg ml$^{-1}$ were prepared in methanol and were stored in refrigerator (−20 °C). 0.2 μg ml$^{-1}$ of finasteride and 1.0 μg ml$^{-1}$ internal standard (IS) were prepared by further diluting stock solutions of finasteride and carbamazepine with methanol as working solutions, which were also stored in refrigerator (−20 °C).

### 2.2. Instrument

The liquid chromatography system (Shimadzu Kyoto, Japan) consisted of a Shimaduz LC-10Advp pump, an SCL-10Advp system controller, a CTO-10Avp column oven, an FCV-10Alvp low-pressure gradient unit, a DGU-14A degasser and an SPD-M10Avp diode array detector. The mass spectrometer was an LCMS-2010 single quadrupole equipped with electrospray ionization interface (Shimaduz, Kyoto, Japan). The samples were dried on Savant drier (Maryland, USA). Vortex mixer was from E&K Scientific (California,USA).The data were collected and processed using LC/MS Solution software.

### 2.3. Chromatographic conditions

The column utilized for chromatographic separation was a Thermo Hypersil-Keystone Hypurity C18 (150 mm × 2.1 mm) analytical column with a particle size of 5 μm. The analytical column was protected by a $C_{18}$ guard-pak cartridge (Waters, Milford, MA, USA). The oven temperature was set at 40 °C. The mobile phase consisted of acetonitrile–water (46:54, v/v) containing 0.1% acetic acid and 0.1% TFA (v/v). With an isocratic HPLC, the mobile phase was eluted at a flow rate of 0.2 ml min$^{-1}$. The wavelength of the SPD-M10Avp diode array detector was set 200–350 nm. The volume of injection was 5 μl.

### 2.4. Mass spectrometer conditions

An LCMS-2010 quadrupole mass spectrometer interfaced with electrospray ionization (ESI) probe was used. ESI, curved desolvation line (CDL) and block temperature were 380, 250

and 210 °C, respectively. Probe voltage was 4.5 kV. Detector voltage was 1.6 kV. CDL voltage was −18 V. Q-array Bios voltage was 45 V. Nebulizing gas flow was 4.5 l min$^{-1}$. Positive selected ion monitor (SIM) mode and $[M+H]^+$s of both finasteride and carbamazepine in SIM mode were chosen for the determination of finasteride. The selected monitoring ions were decided by positive scanning from *m/z* 100 to 500 and the SIM ions were at *m/z* 373 and 237 for finasteride and carbamazepine (IS), respectively. With the help of autotune function of LCMS Solution software (version 2.02), tuning of mass spectrometer was performed by using tuning standard solution (polypropylene glycol). Optimization and calibration were achieved with autotuning.

### 2.5. Plasma sample preparation

0.2 ml plasma sample was placed in a 1.5 ml Eppendorf tube. After the addition of 10 μl of 1.0 μg ml$^{-1}$ internal standard, the tube was briefly vortexed. Then 0.2 ml of acetonitrile was added into the tube. After vortexing for 30 s, the tube was centrifuged at 13,000 rpm for 15 min at 5 °C and the supernatant was transferred to another clear Eppendorf tube. The extract was evaporated to dryness in a Speed Vacplus Model vacuum drier. The residue was dissolved in 100 μl of mobile phase. Then the obtained solution was vortexed for 30 s and centrifuged at 13,000 rpm for 5 min. Five microliters of supernatant was directly injected into the analytical column.

### 2.6. Validation

The method has been validated for selectivity, linearity, precision, accuracy and recovery. This selectivity test was performed by analyzing the blank plasma samples from different sources to test for the interference at the retention time areas of finasteride and IS. Linearity was tested for the range of concentrations 0.2–120.0 ng ml$^{-1}$. In order to calculate the calibration curve, a set of nine non-zero finasteride calibration standards, which were 0.2, 0.5, 2.0, 5.0, 10.0, 20.0, 40.0, 80.0, 120.0 ng ml$^{-1}$, were prepared by diluting standard solution with blank plasma, while internal standard was added to make each standard contain 50 ng ml$^{-1}$ of internal standard. After processed as same as plasma sample preparation, the obtained solutions were detected with LC/ESI-MS. Calibration curve was calculated by plotting peak area ratio ($y$) of finasteride and internal standard against concentrations ($x$, ng ml$^{-1}$). The retention times of IS and finasteride in total ion chromatograms (TIC) were 2.98 and 5.09 min, respectively. The unknown sample concentrations were calculated from the regression equation of the standard curve.

Three quality control samples at concentrations of 0.2, 60.0 and 120.0 ng ml$^{-1}$, which were lowest limit of quantification, medium quality control and high quality control (LLOQ, MQC and HQC) levels, respectively, were prepared by diluting standard solution with blank plasma, and the added amount of internal standard in each quality control sample was also 50 ng ml$^{-1}$. Then quality control samples were processed as same as sample preparation. Sensitivity was determined by analyzing control human plasma in replicates ($n=6$) spiked with the analyte at the lowest level of the calibration curve, 0.2 ng ml$^{-1}$. Intra-day precision and accuracy were evaluated by analyzing each QC sample six times on the same day, while inter-day precision and accuracy were evaluated by analyzing each QC sample in 6 consecutive days.

The recovery of finasteride and IS with acetonitrile was calculated by comparing the peak area response of extracted analytes with unextracted equal amount of standards. Finasteride standards at concentrations of 0.2, 60.0 and 120 ng ml$^{-1}$ were prepared using mobile phase and standard working solution, and the added amount of internal standard was 50 ng ml$^{-1}$. These samples were detected with LC/ESI-MS and the peak area was recorded as $f_s$. Three QC samples (low, medium and high quality control sample) were also detected with LC/ESI-MS and the peak areas were recorded as $f_x$. The recovery of extraction for each finasteride standard and IS was calculated as follow:

$$\text{Recovery of extraction (\%)} = \frac{f_x}{f_s} \times 100\%$$

### 2.7. Pharmacokinetic study

The developed method was used to investigate the plasma concentration–time profile of finasteride after administration of 5 mg dose. Twenty-four healthy volunteers (male) received the investigation. The age of volunteers was between 18 and 25 years (average 22.6 years). The body weight was between 58 and 84 kg (mean 72.4 kg) and the body height was 166–187 cm (mean 172.6 cm). Before test, volunteers must take health exam to ensure all the volunteers have normal liver, heart rate, breath, blood and electrocardiogram. Before and during the 2 weeks for test, volunteers did not take any other medicine. Following written informed consent, volunteers took a 5-mg tablet of finasteride with a little warm boiling water. Drinking and smoking were not allowed, and low fat food was given at 2 h after taking drug. Blood samples were collected in heparinized tubes predose (0 h) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16 and 24 h post-dose. Plasma was immediately separated by centrifugation at 3000 rpm and stored −20 °C until analysis.

## 3. Results and discussion

### 3.1. Optimization of chromatographic conditions and MS conditions

The purpose of the present study was to develop a sensitive and reliable LC/ESI-MS method for the determination of finasteride in human plasma. In the early published HPLC method, usually acetonitrile-potassium dihydrogenphosphate water solution or acetonitrile–water [11–14] were used as mobile phase, and no appropriate inter standard was used. In a preliminary study, it was found that separation of finasteride and carbamazepine were achieved on a Thermo Hypersil-Keystone Hypurity C18 analytical column by using the mobile phase consisting of acetonitrile–water. The separation and ionization of finasteride and carbamazepine were affected by the components of mobile phase. Both finasteride and carbamazepine were easily

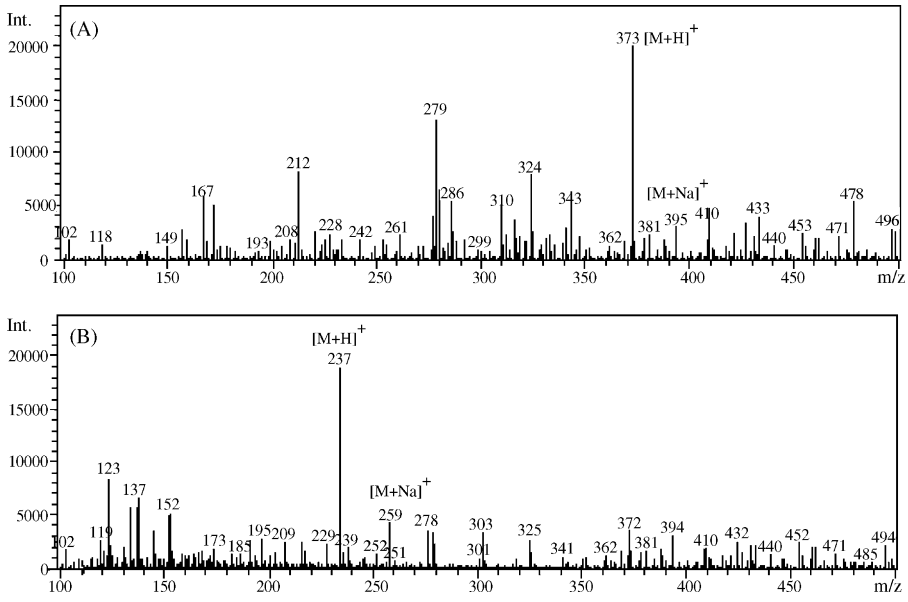



Fig. 2. LC/ESI-MS mass spectra of finasteride and carbamazepine in positive scan mode from *m/z* 100 to 500. Chromatographic conditions and MS parameters were described in Sections 2.3 and 2.4. (A) Finasteride and (B) carbamazepine.

protonized in a stronger acidic mobile phase before entering the ionization ambient (chamber). In our preliminary experiment, the results showed that using the mixture of acetic acid and TFA to adjust pH and function as modifier was better than only using acetic acid or TFA. Separation conditions such as percentage of water, acetonitrile, acetic acid and TFA were optimized. Both the separation and ionization of finasteride were affected most by the percentages of acetic acid and TFA. The retention times of finasteride and IS were also affected by the acidity of mobile phase. When acidity of mobile phase was increased, the retention times of finasteride and IS were decreased. However, the sensitivity of finasteride was improved by increasing acidity of mobile phase because of raising the rate of ionization. Considering both separation and ionization of finasteride, the acidity containing 0.1% acetic acid and 0.1% TFA (v/v) in mobile phase was appropriate for separation and ionization of finasteride. The optimized chromatographic conditions were listed in Section 2.3.

In order to select an appropriate ionization mode in LC/MS analysis, the mass spectra were measured in both ESI and APCI mode by scanning from 100 to 500 and injecting finasteride and internal standard solutions. The base peak intensities of positive ion were higher than those of negative ion in both ionization modes. ESI mode exhibited a more intensive peak than APCI mode did, so the positive ion ESI mode was selected in this study. The positive ion mass spectra of finasteride and internal standard in scan mode were shown in Fig. 2. As shown in Fig. 2, both finasteride and IS were characterized by a protonated molecular ion $[M+H]^+$ (at *m/z* 373 and 237, respectively) as base peak. SIM mode involved the use of the $[M+H]^+$ ions at *m/z* 373 (finasteride) and 237 (IS) was chosen for the quantitative analysis of finasteride. The optimized ESI-MS conditions were listed in Section 2.4. Then, a very simple, fast, sensitive and selective LC/MS method to quantify the finasteride was developed. Selective and total ion chromatograms of finasteride and carbamazepine (IS) were shown in Fig. 3. The retention times of IS and finasteride in TIC were 2.98 and 5.09 min, respectively.

### 3.2. Selectivity and matrix effect

The LC/ESI–MS method has high selectivity because only selected ions from the analytes of interest were monitored. Comparison of the chromatograms of the blank and the spiked human plasma samples (see Fig. 3) indicated no significant interferences at the retention time areas of the analyte and the IS. As shown from it, finasteride and IS exhibited favorable selectivity under the optimized conditions.

It was very important to investigate the matrix effects to develop a reliable and reproducible LC/ESI–MS method. Here, the matrix effect was evaluated by the following experiments. Finasteride and carbamazepine were spiked separately into human blank plasma as well as into the mobile phase. After prepared with exactly the same procedures as described in the Section 2.5, these samples were detected by LC/ESI-MS. The results indicated that there was no significant difference in peak areas of finasteride and IS between six different drug-free human plasma samples and the mobile phase. It showed that no endogenous compounds significantly influenced the ionization of finasteride and IS.

### 3.3. Linearity

The calibration curve, which was linear in the range of 0.2–120 ng ml$^{-1}$, was $y = 0.0462 + 0.03248x$ with correlation coefficients being 0.9987. A crucial problem was regarding the sensitivity so that it can be used for the determination of the very low concentration of finasteride after administration of low dosage in human plasma. The method reported here

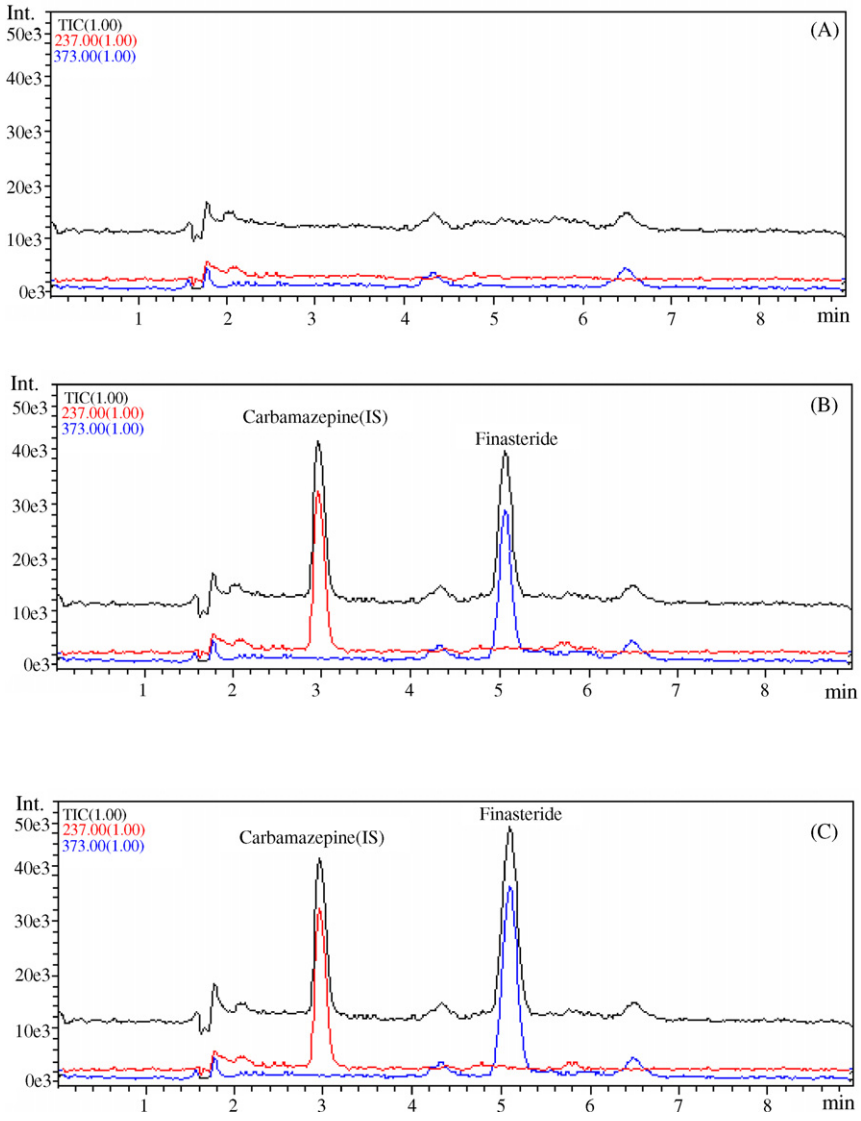



Fig. 3. Selective and total ion chromatograms of finasteride and carbamazepine (IS). Selected positive monitoring ions were at *m/z* 237 (carbamazepine, IS) and 373 (finasteride); other conditions were the same as in Fig. 2; (A) blank plasma, (B) blank plasma spiked with standard (30 ng ml$^{-1}$) and IS, and (C) human plasma sample after administration of finasteride and spiked with IS.

was very sensitive due to using optimal ESI-MS conditions and the advantages of LC/ESI-MS in the SIM mode. When the developed method was applied to clinical samples, the limit of quantification (LOQ) was very important. The lowest standard concentration in the calibration curve was considered as the lower limit of quantification (LLOQ), which was 0.2 ng ml$^{-1}$. For LLOQ, the mean percentage deviation from the nominal concentration was 8.5 % and precision was 11.2%. A good signal-to-noise ratio (10:1) was observed at the LLOQ indicating that the corresponding valve could be reached. The sensitivity of the developed method was higher than early published HPLC methods [11–14] and polarography [15]. The sensitivity of the developed method was also comparable with early reported LC/MS-MS method [16,17], which was the highest sensitivity among all the earlier published methods. However, the preparation procedure of human plasma by one-step protein precipitation was much simpler than SPE and liquid–liquid extraction methods.

### 3.4. Precision, accuracy and recovery

Both the intra- and inter-day accuracy and precision of the developed method were determined by six replicate analyses of quality control samples containing known concentrations of finasteride range from 0.2 to 120.0 ng ml$^{-1}$. The precision of the method was described as relative standard deviation (R.S.D.) among each assay. The accuracy (R.E.) was described as a percentage error of measured concentrations versus nominal concentrations of finasteride in QC samples. The results of intra- and inter-day accuracy and precision were listed in Table 1. The intra-assay precision and accuracy of quality control samples were in the order of 2.1–11.2% and

Table 1
Intra- and inter-day precision and accuracy of quality control samples for the determination of finasteride in human plasma

| Added concentration (ng ml$^{-1}$) | Mean found concentration (ng ml$^{-1}$) | R.E.[a] (%) | R.S.D. (%, $n=6$) |
|---|---|---|---|
| Intra-day | | | |
| 0.200 | 0.217 | 8.5 | 11.2 |
| 60.000 | 60.502 | 0.84 | 3.4 |
| 120.000 | 118.471 | −1.3 | 2.1 |
| Inter-day | | | |
| 0.200 | 0.223 | 11.5 | 12.1 |
| 60.000 | 59.104 | −1.5 | 4.2 |
| 120.000 | 124.116 | 2.6 | 3.4 |

[a] R.E. was mean relative error of six replicate analyses.

−1.3% to 8.5%, respectively. The inter-day assay precision and accuracy were in the range of 3.4–12.1% and −1.5% to 11.5%, respectively. Absolute recoveries of finasteride at three QC levels were measured by assaying the samples as described in Section 2.6 and comparing the peak areas of both finasteride and IS with those obtained from direct injection of the compounds dissolved in the mobile phase. The absolute recovery of finasteride, determined at three concentrations (0.2, 60 and 120 ng ml$^{-1}$), were 85.7%, 89.1% and 86.3%, respectively. The recovery of IS was investigated as 74.9%. The extraction recoveries of IS from human were somewhat lower than that of finasteride, which may be caused by the loss of analyte when it was transferred. However, the determination and quantification of finasteride were not affected by the recovery in any case.

### 3.5. Stability

Stability was evaluated as a part of method validation. Finasteride standards at concentrations of 0.2, 60.0 and 120.0 ng ml$^{-1}$ (LLQC, MQC and HQC) were used for stability experiments. Ten milliliters of 0.2, 60.0 and 120.0 ng ml$^{-1}$ finasteride standard solution were prepared by diluting standard solution with blank plasma, and the added amount of internal standard in each quality control sample was also 50 ng ml$^{-1}$. The results indicated that the difference of measured concentration from time 0 to 8 h was less than 6.4% when these samples were placed at room temperature, which allowed us to conclude that processed samples were stable for at least 8 h. When these standards were stored at −20 °C, four thaw and freeze cycles were performed after processed as Section 2.5. During each cycle, 1ml standard were processed. The difference of measured concentration from nominal concentration was less than 5.8% for 0.2, 60.0 and 120.0 ng ml$^{-1}$, and the results indicated that the stability of finasteride was not affected by freezing and thawing because of no significant substance loss during repeated thawing and freezing. In long-term stability experiments, after storage for 1 month at −20 °C, more than 94.2% of finasteride remained according to their peak areas at each concentration. This demonstrated that these finasteride standards were stable for at least 30 days when they were stored at −20 °C.

Stability of processed samples was also performed. Processed quality control samples were used for this aim. The processed samples were analysed in 1 day and left at ambient temperature. Then the samples were analysed in following days using freshly prepared calibration standards. The results were listed in Table 2. The results showed that the processed samples were stable at least 2 days at room temperature if the allowed difference between the found and nominal concentration were 10%. After these processed QC samples were stored for 1 month at −20 °C, more than 93.8% of finasteride remained according to their peak areas at each concentration. This showed that processed samples were stable for at least 30 days when they were stored at −20 °C.

Table 2
Stability of processed samples

| Nominal concentration (ng ml$^{-1}$) | Time (day) | Found concentration (ng ml$^{-1}$) | R.E.[a] (%) | R.S.D. (%) |
|---|---|---|---|---|
| 0.200 | 0 | 0.213 | 6.5 | 11.2 |
| | 1 | 0.192 | −4.0 | 10.8 |
| | 2 | 0.183 | −8.5 | 11.5 |
| 60.000 | 0 | 60.536 | 0.9 | 3.4 |
| | 1 | 58.192 | −3.0 | 3.8 |
| | 2 | 56.827 | −5.3 | 4.1 |
| 120.000 | 0 | 118.104 | −1.6 | 1.9 |
| | 1 | 115.892 | −3.4 | 2.2 |
| | 2 | 113.246 | −5.6 | 2.4 |

[a] R.E. was mean relative error of six replicate analyses.

### 3.6. Application

The developed method was successfully used for the determination of plasma concentrations of finasteride after oral administration of a 5 mg dose to 24 healthy volunteers. Fig. 3(C) was LC/ESI-MS TICs of a human plasma sample after administration of finasteride. Fig. 4 showed mean ± S.D. plasma concentration–time profile of finasteride. The pharmacokinetic parameters were shown in Table 3. The obtained values were consistent with previously published reports [10], which indicated a suitability of the analytical method for pharmacokinetic studies.

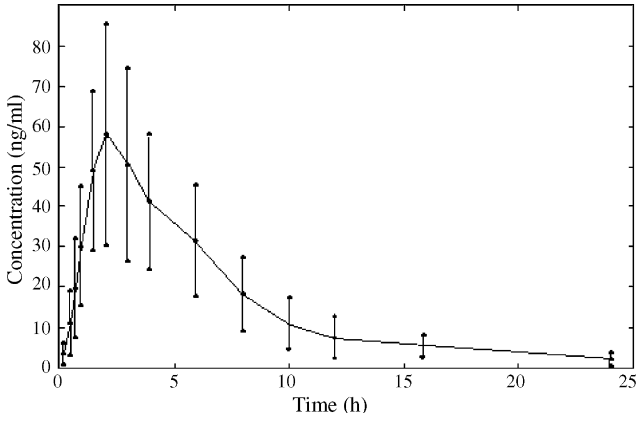



Fig. 4. Plasma concentration–time profile (mean ± S.D.) of finasteride for 24 healthy volunteers following oral administration of a single 5-mg finasteride tablet.

Table 3
Mean (±S.D.) pharmacokinetic parameters for finasteride in healthy volunteers ($n = 24$)

| | |
|---|---|
| $T_{max}$ (h) | 1.8 ± 0.4 |
| $T_{1/2}$ (h) | 5.7 ± 5.2 |
| $C_{max}$ (ng ml$^{-1}$) | 56.4 ± 8.7 |
| $AUC_{0\rightarrow 96}$ (h ng ml$^{-1}$) | 286.8 ± 41.5 |
| CLF (l h$^{-1}$) | 8.5 ± 1.6 |

## 4. Conclusions

A fast, sensitive, specific LC/ESI-MS method for the determination of finasteride in human plasma was developed and validated. Compared with the previously published methods, significantly lower limit of quantification, which was 0.2 ng ml$^{-1}$, was achieved in plasma. By use of 0.2 ml plasma sample and deprotienation with acetonitrile, the steps of sample preparation were very simple. The method has been successfully applied to the pharmacokinetics studies and satisfactory results were obtained, which demonstrates that the method is reproducible, sensitive and reliable.

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## References

[1] T. Liang, M.A. Cascieri, A.H. Cheung, G.F. Reynolds, G.H. Rasmusson, Endocrinology 117 (1985) 571–579.
[2] J.R. Brooks, C. Berman, R.L. Primka, G.F. Reynolds, G.H. Rasmusson, Steroids 47 (1986) 1–19.
[3] A.B. Barqawt, J.W. Moul, A. Ziada, L. Handel, E.D. Crawford, Urology 62 (2003) 872–876.
[4] J.K. Mellon, Eur. J. Cancer 41 (2005) 2016–2022.
[5] E.A. Klein, Crit. Rev. Oncol. Hematol. 54 (2005) 1–10.
[6] A.G. Lekas, A.C. Lazaris, M. Chrisofos, A.G. Papatsoris, D. Lappas, E. Patsouris, C. Deliveliotis, Urology 68 (2006) 436–441.
[7] C. Roehrborn, J.P.W. Heaton, Eur. Urol. Suppl. 5 (2006) 716–721.
[8] G. Novara, A. Galfano, M. Gardi, V. Ficarra, L. Boccon-Gibod, W. Artibani, Eur. Urol. Suppl. 5 (2006) 418–429.
[9] F.K. Habib, M. Ross, R. Tate, G.D. Chisholm, Clin. Endocrinol. 46 (1972) 137–144.
[10] D.H. Peters, E.M. Sorkin, Drugs 46 (1993) 177–208.
[11] T. Takano, S. Hata, J. Chromatogr. B 676 (1996) 141–146.
[12] G. Carlucci, P. Mazzeo, J. Chromatogr. B 693 (1997) 245–248.
[13] P. Ptacek, J. Macek, J. Klýma, J. Chromatogr. B 738 (2000) 305–310.
[14] A.A. Syed, M.K. Amshumali, J. Pharmaceut. Biomed. Anal. 25 (2001) 1015–1019.
[15] S.M. Amer, Il Farmaco 58 (2003) 159–163.
[16] M.L. Constanzer, C.M. Chavez, B.K. Matuszewski, J. Chromatogr. B 658 (1994) 281–287.
[17] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882–889.
[18] F.G. de Menezes, W. Ribeiro, D.R. Ifa, M.E. de Moraes, M.O. de Moraes, G. de Nucci, Arzneimittelforschung 51 (2001) 145–150.
[19] A. Guarna, G. Danza, G. Bartolucci, A. Marrucci, S. Dini, M. Serio, J. Chromatogr. B: Biomed. Appl. 674 (1995) 197–204.
[20] J. Macek, J. Chromatogr. B 764 (2001) 207–215.