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Short communication

# Rapid determination of finasteride in human plasma by UPLC–MS/MS and its application to clinical pharmacokinetic study

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ABSTRACT

A rapid, specific, and sensitive method utilizing reversed-phase ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) was developed and validated to determine finasteride levels in human plasma. The plasma samples were prepared by liquid–liquid extraction with ethyl acetate, evaporation, and reconstitution. MS/MS analyses were performed on a triple–quadrupole tandem mass spectrometer by monitoring protonated parent → daughter ion pairs at *m/z* 373 → 305 for finasteride and *m/z* 237 → 194 for carbamazepine (internal standard, IS). The method was validated with respect to linearity, recovery, specificity, accuracy, precision, and stability. The method exhibited a linear response from 0.1 to 30 ng/mL ($r^2 > 0.998$). The limit of quantitation for finasteride in plasma was 0.1 ng/mL. The relative standard deviation (RSD) of intra- and inter-day measurements was less than 15% and the method was accurate within −6.0% to 2.31% at all quality-control levels. The mean extraction recovery was higher than 83% for finasteride and 84% for the IS. Plasma samples containing finasteride were stable under the three sets of conditions tested and the processed samples were stable up to 29 h in an autosampler at 5 °C. Detection and quantitation of both analytes within 3 min make this method suitable for high-throughput analyses. The method was successfully applied to a pharmacokinetic study of finasteride in healthy volunteers following oral administration.

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## 1. Introduction

Benign prostate hyperplasia (BPH) is an androgen-dependent, nonmalignant growth of the periurethral prostate gland that results in enlargement of the prostate gland and urinary obstruction [1]. Prostate cancer, the most common cancer among men over 50 years with increasing prevalence with age, is the leading cause of cancer death [1,2]. Finasteride (CAS 98319-26-7), a member of the 4-azasteroid family, is a potent inhibitor of 5-alpha-reductase, which synthesizes androgen dihydrotestosterone (DHT) through the reduction of testosterone [3]. Increased levels of DHT are responsible for maintaining growth in the development of prostrate cancer and BPH [4]. Chemotherapeutic treatment with finasteride has shown a beneficial effect in the prevention of prostate cancer [5], as finasteride is rapidly absorbed and widely distributed in the body after oral administration. Therefore, developing an assay that is both sensitive and specific for finasteride in human biofluids is essential.

The quantitation of finasteride in biological samples has been performed with several methods including high-performance liquid chromatography (HPLC) [6–8], isotope-dilution mass spectrometry [9], polarography [10], spectrophotometry [11], and high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) [12–18]. Although HPLC is a well-established technique, it suffers from poor sensitivity and specificity. Previous methods have involved multistep sample preparations such as solid-phase extraction [8,18] and run times up to 38 min [6]. The addition of tandem MS/MS significantly enhances the sensitivity and specificity [13], but the chromatographic run times are still too long for high-throughput analyses [12,14–16]. Also, one of these methods [16] uses trifluoroacetic acid as mobile phase additive, which is reported to suppress electrospray ionization signals of analyte ions [19]. Hence, there is need of more rapid and sensitive method for high-throughput bioanalytical applications.

UPLC techniques offer efficient chromatography with reduced run times and improved sensitivity [20] by taking advantage of smaller particle size (1.7 μm) and higher operating pressures than

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conventional HPLC. UPLC techniques have been explored for applications in pharmacokinetic analyses [21], drug metabolism [22], and metabolite profiling [23]. To the best of our knowledge, the use of UPLC–MS/MS has not been demonstrated for pharmacokinetic studies of finasteride.

The current study describes a validated UPLC–MS/MS method for quantification of finasteride in human plasma that boasts higher specificity, improved resolution, increased sensitivity (0.1 ng/mL), and faster analysis times (3.5 min) over conventional HPLC–MS/MS. Even the recently reported faster LC–MS methods [12,16] were also have longer chromatographic run times (up to 13 min) with lesser sensitivity (0.2–1 ng/mL) than our method. The utility of the current method in pharmacokinetic analyses was also demonstrated.

## 2. Experimental

### 2.1. Reagents and chemicals

Finasteride (Lot No. 075K4712) and carbamazepine (Lot No. 036K1219) were purchased from Sigma (St. Louis, MO, USA). Finasteride for oral administration used in this study was supplied by MSD Korea Co. Ltd. (Seoul, Korea). Acetonitrile and ethyl acetate (HPLC-grade) were obtained from Merck (Darmstadt, Germany). ACS reagent-grade ammonium formate, formic acid, and other chemicals were purchased from Sigma. Blank human plasma samples were obtained from healthy Korean male volunteers. All solvents were filtered through a 0.22-μm membrane filter (Millipore, Dublin, Ireland) and degassed. Water for chromatography was purified by a Milli-Q water purification system (Millipore).

### 2.2. Liquid chromatography

Analytical separations were performed with an ACQUITY™ UPLC system equipped with a micro-vacuum degasser, thermostatted autosampler, binary gradient pumps, thermostatted column compartment, and an ACQUITY™ UPLC BEH $C_{18}$ Column (50 mm × 2.1 mm, 1.7 μm), all obtained from Waters Corp. (Milford, MA, USA). The column temperature was maintained at 25 °C. An isocratic mobile phase consisting of a 58:42 (*v*/*v*) mixture of 1 mM ammonium formate buffer at pH 3.0 (adjusted with formic acid) and acetonitrile was used at flow rate of 0.2 mL/min. The mobile phase was filtered through a 0.22-μm membrane filter (Millipore) before use. The autosampler temperature was kept at 5 °C and the samples were injected onto the column with an injection volume of 5 μL (partial loop in needle overfill mode). The data acquisition run time was kept at 3.5 min for the mass spectrometer (MS). All data were collected and processed using MassLynx™ software with QuanLynx™ (Waters Corp.).

### 2.3. Mass spectrometry (MS)

Mass spectra were acquired on a Quattro Premier XE™ Micromass® triple quadrupole mass spectrometer (Waters Corp.) with an electrospray ionization interface in positive ionization mode. The optimal MS parameters were as follows: source temperature 80 °C, desolvation temperature 250 °C, capillary voltage 3.5 kV, cone voltage 57.0 V, cone gas flow 50 L/h, desolvation gas flow 900 L/h. Nitrogen was used as the cone and desolvation gas. Multiple-reaction monitoring (MRM) analyses were performed using a collision gas (argon) at $2.8 \times 10^{-3}$ mbar. Quantitation was performed using the following MRM transitions: $m/z$ 373 → 305 for finasteride and $m/z$ 237 → 194 for carbamazepine with a dwell time 0.10 s per transition. The optimized collision energy of both finasteride and the IS was 41.0 and 25 eV, respectively.

### 2.4. Preparation of standards and quality controls

Standard 1 mg/mL stock solutions of finasteride and the IS were prepared separately in methanol. Standard working solutions of finasteride at concentrations of 1, 2, 10, 50, 100, 200, and 300 ng/mL, and a 10 ng/mL solution of IS, were prepared by serial dilution of stock solutions. The diluent consisted of a mixture of 1 mM ammonium formate buffer at pH 3.0 and acetonitrile (50/50, *v*/*v*). Drug-free plasma was spiked with standard solutions to prepare calibration standards with final concentrations of 0.1, 0.2, 1, 5, 10, 20, and 30 ng/mL of finasteride. Quality-control (QC) samples containing finasteride were prepared in a similar manner at four concentration levels: 0.1 ng/mL (lower limit of quantitation, LLOQ), 0.2 ng/mL (low, LQC), 5 ng/mL (middle, MQC), and 20 ng/mL (high, HQC). All standard stock solutions were kept at −20 °C prior to analysis. Standards and quality controls were extracted daily before analysis using the procedure described below for plasma samples.

### 2.5. Plasma sample preparation

Plasma samples were stored at −80 °C and allowed to thaw gradually to room temperature before processing. After transferring 500-μL aliquots of plasma into 15.0-mL glass tubes, 20 μL of IS solution (10 ng/mL of carbamazepine) was added to each tube and the tubes were briefly vortexed. Five milliliters of ethyl acetate was added to each tube and the mixture was vortexed for 10 min and centrifuged at 3500 rpm for 10 min at 5 °C. Then, 4.5 mL of the upper organic layer was transferred to another glass tube and evaporated to dryness for 80 min at 45 °C using a SpeedVac vacuum evaporator (Savant Instruments, Holbrook, NY, USA).

The dry residue was reconstituted in 100 μL of diluent and vortexed for 1 min. The resulting solution was filtered through a PVDF filter (0.2 μm, 4 mm; Millipore) into glass vials and 5 μL of this solution was injected into the UPLC–MS/MS system.

### 2.6. Method validation

The method described above was validated with regard to linearity, specificity, accuracy, precision, percent recovery, and stability according to accepted guidelines [24,25].

#### 2.6.1. Linearity and sensitivity

Calibration curves were prepared daily prior to sample analysis by analyzing calibration standards ranging in concentration from 0.1 to 30 ng/mL. Calibration curves were plotted using the peak area ratio of finasteride to the IS versus the nominal concentration. Continuous calibration curves were fitted to the raw data by a weighted ($1/x$) least squares regression.

Sensitivity was defined by the lower limit of quantitation (LLOQ), which was the concentration of finasteride at which the signal to noise (S/N) ratio was greater than 10 with acceptable accuracy and precision. This value was set as the lowest concentration in calibration curves.

#### 2.6.2. Precision and accuracy

Intra-day accuracy and precision were determined by replicate analyses ($n = 5$) of each of the four QC samples (0.1, 0.2, 5, and 20 ng/mL) performed on the same day. Inter-day accuracy and precision were determined by replicate analyses ($n = 5$) of the same QC concentrations on five different days. Accuracy was calculated as the percent deviation (% DEV) of the mean observed concentration from the nominal concentration according to the below expression [26]:

$$\text{DEV}(\%) = \left[\frac{\text{Nominal concentration} - \text{Mean observed concentration}}{\text{Nominal concentration}}\right] \times 100$$

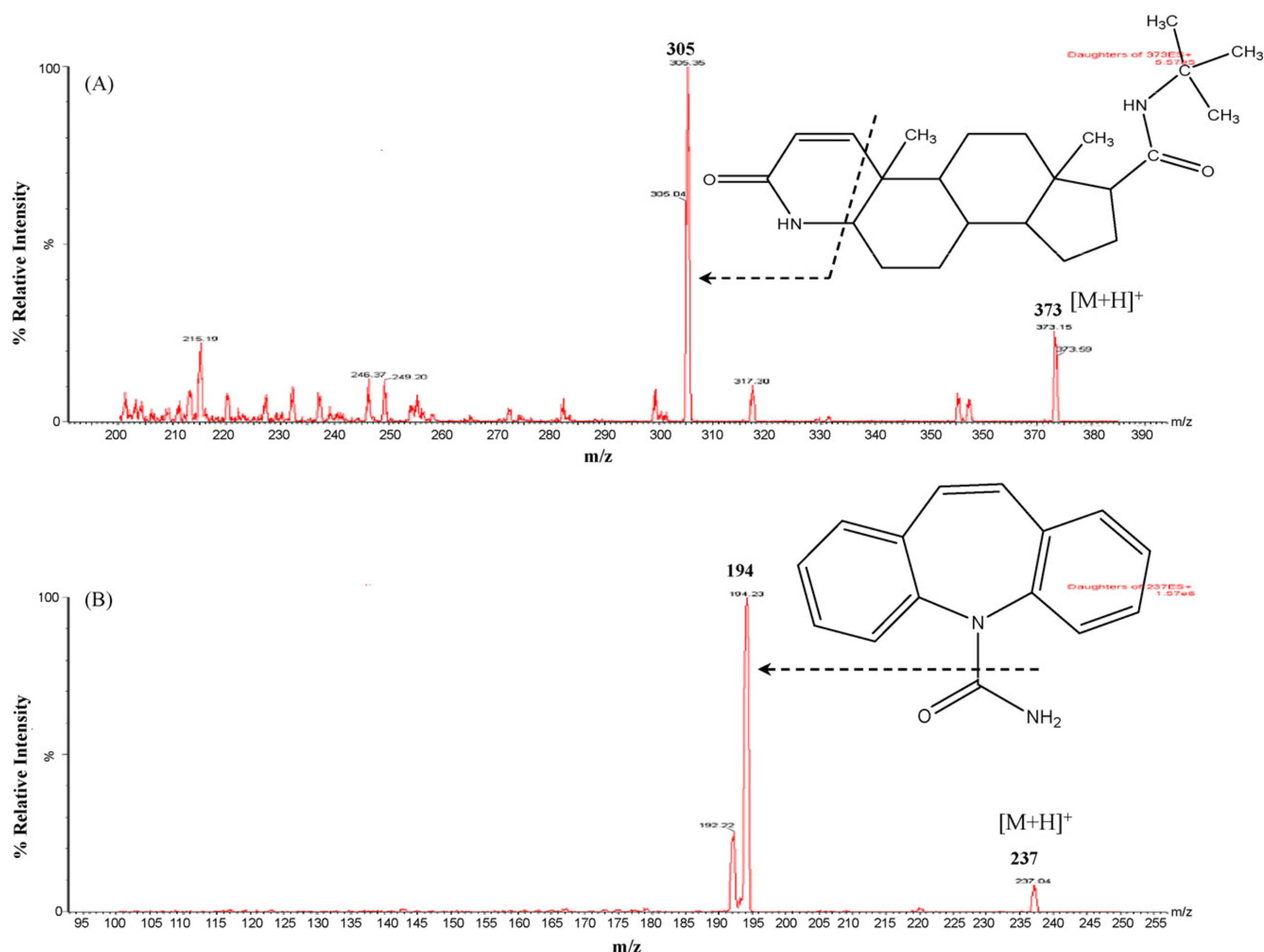


**Fig. 1.** Product ion mass spectra are given for (A) finasteride and (B) carbamazepine.

The precision of the method was determined by the relative standard deviation (RSD) as defined by

$$\mathrm{RSD}(\%) = \left[\frac{\mathrm{SD}}{M}\right] \times 100,$$

where SD is the standard deviation and *M* is the mean observed concentration of replicate analyses.

*2.6.3. Specificity*

Specificity tests were performed by a comparison of MRM chromatograms obtained from drug-free plasma samples from six healthy volunteers with each respective plasma spiked using finasteride (0.1 ng/mL) and the IS (10 ng/mL).

*2.6.4. Extraction recovery*

The percent extraction recovery of finasteride was determined by triplicate analyses of four QC samples and was calculated as the peak area ratio of finasteride from plasma samples spiked with a known concentration of finasteride to that of unextracted samples that had been spiked with the same finasteride concentration after extraction of the blank plasma. The percent recovery of IS was calculated in a similar manner.

*2.6.5. Stability*

The stability of plasma QC samples at low (0.2 ng/mL) and high (20 ng/mL) concentrations was determined in triplicate by comparing the peak area ratios of freshly prepared samples with those obtained after stability testing. The stability of finasteride was evaluated under four different conditions: post-preparative stability at 5 °C for 29 h, freeze and thaw stability for three cycles, short-term temperature stability at room temperature for 6 h, and long-term temperature stability at −20 °C for 64 days. The stability of stock solutions of finasteride and carbamazepine were evaluated at −20 °C for 38 days after diluting each with mobile phase to a concentration of 100 ng/mL and comparing their peak areas to those of freshly prepared samples at the same nominal concentration.

*2.7. Pharmacokinetic applications*

The method was successfully applied in a pilot pharmacokinetic study of finasteride to determine the plasma concentrations of finasteride after administration of a 1-mg dose. The study protocol was approved by the Institutional Review Board of Kyungpook National University Hospital, Daegu, Korea. Six healthy Korean male volunteers aged 24.8 ± 2.3 years with a mean weight of 71.18 ± 9.9 kg participated in this study. During the study period, volunteers did not take any other medications and were hospitalized as inpatients. After giving their written informed consent, all volunteers were orally administered a single tablet containing a 1-mg dose of finasteride. Blood samples (6 mL) were collected in sodium heparinized tubes before (0 h) and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 h after the dose. Plasma from all blood samples was immediately separated by centrifugation at 3000 rpm for 10 min. All plasma samples were stored at −80 °C until analysis.

## 3. Results and discussion

### 3.1. Mass spectrometry (MS)

Measurements of finasteride and IS levels in samples of human plasma were made with a UPLC–MS/MS instrument in MRM scan mode. Solutions of finasteride and IS (1 μg/mL) were directly infused into mass spectrometer along with mobile phase (0.2 mL/min) and MS parameters were optimized to get maximum sensitivity for respective product ions. The optimized mass spectrum of finasteride shows an intense peak corresponding to the $[M+H]^+$ ion at *m/z* 373 and the IS mass spectrum contains an intense peak at *m/z* of 237 corresponding to the $[M+H]^+$ ion. The fragmentation patterns of these ions under these conditions contained intense product peaks at *m/z* 305 for finasteride and another intense product ion at *m/z* 194 for IS (Fig. 1). Therefore, the corresponding transitions associated with these product peaks were selected for MRM analysis.

### 3.2. Chromatography

In previously published LC–MS methods, acetonitrile-acidic buffers consisting of trifluoracetic acid [16] and ammonium acetate [12] were used as the mobile phase. However, in preliminary optimization trials, the use of trifluoracetic acid reduced the MS sensitivity and acetate buffers were more suitable in negative MS ionization mode. Therefore, an ammonium formate buffer acidified with formic acid (pH 3), which increased the method sensitivity in positive mode through assisting ionization of $[M+H]^+$ ions of both finasteride and the IS, was used herein. The pH 3 mobile phase was optimal for both MS sensitivity and chromatographic separation. The UPLC technique, with smaller column particle size (1.7 μm), separated finasteride and the IS from the sample matrix within 3 min, significantly faster than previous LC–MS methods [12,14–16].

Although, carbamazepine used in as an IS in previous report [16] is not an analogue of finasteride and other internal standards as summarized in the review article [17], are available. However, throughout the study we found carbamazepine suitable as IS, as proved by validation data.

### 3.3. Sample preparation and recovery

Although, use of solid-phase extraction procedures reduces the matrix effect considerably, it increases overall time and cost of analysis. In present study, we optimized simple liquid–liquid extraction procedure which is fast enough for high-throughput analysis.

The mean percent recovery ($n = 3$) of finasteride from plasma at QC concentrations (0.2, 5, 20 ng/mL) ranged from 84.0% to 86.4% and the mean percent recovery of the IS at a concentration 10 ng/mL was 84.6% with an acceptable precision (% RSD < 10). These results suggest that developed sample preparation procedure is acceptable for extraction of finasteride and IS from plasma samples.

### 3.4. Specificity

Typical MRM chromatograms obtained from the drug-free plasma, plasma spiked with standard finasteride (0.1 ng/mL) and IS (10 ng/mL), and plasma samples obtained 1 h after oral administration of finasteride and spiked with IS (10 ng/mL) are shown in Fig. 2. Retention times of finasteride and the IS were 2.5 and 1.4 min, respectively. No interference from endogenous peaks was observed at these retention times.

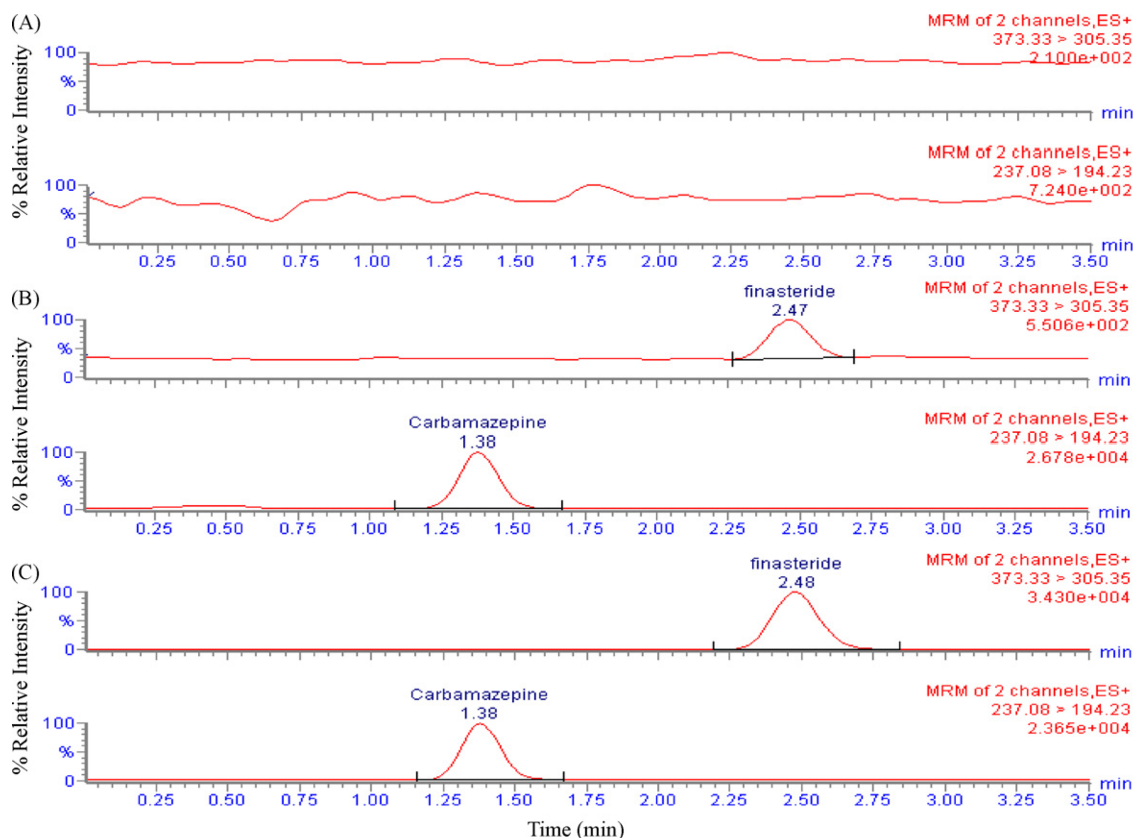


**Fig. 2.** Typical MRM chromatograms of finasteride (upper panel) and carbamazepine (lower panel) in human plasma samples obtained from (A) a drug-free blank plasma sample, (B) a plasma sample spiked with finasteride at the LLOQ (0.1 ng/mL) with 10 ng/mL carbamazepine, and (C) a plasma sample from a volunteer 1 h after the administration of a 1-mg finasteride tablet spiked with carbamazepine. (Experimental conditions were same as mentioned in text).

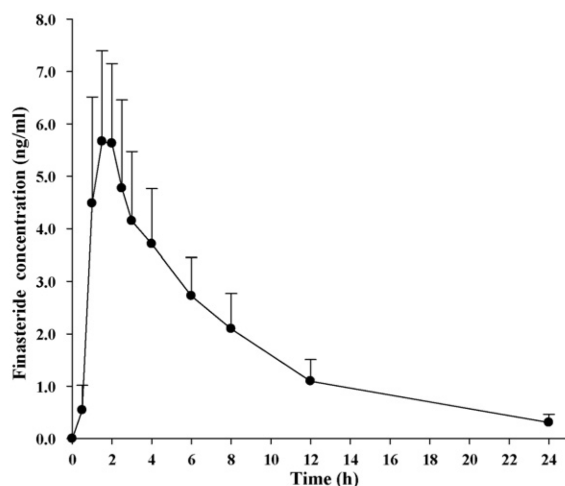


**Fig. 3.** Mean finasteride plasma concentration as a function of time after a single oral dose 1-mg finasteride for 6 healthy volunteers. The vertical bars show the positive standard error.

### 3.5. Lower limit of quantitation (LLOQ) and linearity

The lower limit of quantitation was 0.1 ng/mL with a precision (% RSD) and accuracy (% DEV) of 13.7% and −4.00%, respectively. Calibration curves for finasteride in plasma were linear from 0.1 to 30 ng/mL, as shown by the mean correlation coefficient ($r^2$) of 0.998 ($n$ = 5) obtained for intra-day analyses. The line equation for this calibration curve was $y = 0.146x - 0.00607$, where $y$ is the peak area ratio of finasteride to the IS and $x$ is the concentration of finasteride in ng/mL. Improved chromatographic efficiency of UPLC gives better sensitivity to the method (0.1 ng/mL) than that of pervious studies using LC–MS [12,14–18].

As, maximum plasma concentration observed during pharmacokinetic study was 8 ng/mL for 1-mg dose (Fig. 3), calibration curve of range 0.1–20 ng/mL can also be suitable. However, concentrations calculated from both calibration curves using these ranges (0.1–30 and 0.1–20 ng/mL), showed no significant difference (<5% deviation) and both ranges were comparable (see Supplementary Information).

### 3.6. Precision and accuracy

Intra-day and inter-day accuracy and precision are summarized in Table 1. The intra-day and inter-day precision values (% RSD) for the various concentrations ranged from 4.21% to 15.0% and 3.91% to 13.2%, respectively. Intra-day and inter-day accuracy values ranged from −4.0% to 2.50% and from −6.00% to 1.50%, respectively. Both accuracy and precision were found to be acceptable for bioanalytical applications.

### 3.7. Stability

The stability of finasteride in human plasma was measured in triplicate at finasteride concentrations of 0.2 and 20 ng/mL as a part of the method validation. As shown in Table 2, the finasteride standards were stable under all of the evaluated conditions. The finasteride and IS stock solutions were found to be stable up to 38 days at −20 °C (see Table 2).

### 3.8. Pharmacokinetic application

The validated UPLC–MS/MS method was successfully applied to a pharmacokinetic study. Plasma concentrations of finasteride were determined after a single 1-mg oral dose of finasteride was administered to six healthy Korean male volunteers. Fig. 3 shows the mean plasma concentration of finasteride as a function of time after administration and based on these data pharmacokinetic parameters were estimated using Pharsight WinNonlin 5.2 software. Mean (±SD) pharmacokinetic parameters obtained were, AUC (area under plasma concentration–time curve) 43.83 (±9.03) h ng/mL and maximum concentration ($C_{max}$) of 6.39 (±1.54) ng/mL was reached in 1.9 h ($T_{max}$). These pharmacokinetic parameters were in accordance with previously published reports [27].

**Table 1**
The intra-day and inter-day precision and accuracy of quality-control samples containing finasteride at four concentrations (0.1, 0.2, 5, and 20 ng/mL) in plasma (weight: 1/$x$).

| Added concentrations (ng/mL) | Calculated concentrations ($n$ = 5, mean ± SD, ng/mL) | | Precision (% RSD) | | Accuracy (% DEV) | |
|---|---|---|---|---|---|---|
| | Intra-day | Inter-day | Intra-day | Inter-day | Intra-day | Inter-day |
| 0.1 | 0.104 ± 0.014 | 0.106 ± 0.014 | 13.7 | 13.2 | −4.00 | −6.00 |
| 0.2 | 0.198 ± 0.030 | 0.199 ± 0.021 | 15.0 | 10.7 | 1.00 | 0.50 |
| 5 | 4.94 ± 0.208 | 5.04 ± 0.197 | 4.21 | 3.91 | 1.20 | −0.80 |
| 20 | 19.5 ± 1.375 | 19.7 ± 1.394 | 7.04 | 7.08 | 2.50 | 1.50 |

**Table 2**
Results of the stability of finasteride under four different conditions and stock solution stability of finasteride and IS.

| Added concentrations (ng/mL) | | Calculated concentrations (mean ± SD, ng/mL) | | | | |
|---|---|---|---|---|---|---|
| | | Day 0 | Post-preparation[a] | Freeze–thaw cycles ($n$ = 3) | Short-term[b] | Long-term[c] |
| 0.20 | Mean ($n$ = 3) ± SD | 0.191 ± 0.016 | 0.185 ± 0.013 | 0.164 ± 0.01 | 0.196 ± 0.029 | 0.178 ± 0.006 |
| | % Relative concentration | – | 96.9 | 85.9 | 102.6 | 93.2 |
| 20 | Mean ($n$ = 3) ± SD | 18.5 ± 0.503 | 18.6 ± 1.001 | 19.1 ± 0.720 | 19.6 ± 0.879 | 19.4 ± 1.194 |
| | % Relative concentration | – | 100.5 | 103.2 | 105.9 | 104.9 |
| 100 (Finasteride stock) | % Relative concentration[d] | | | 97.1 | | |
| 100 (IS stock) | % Relative concentration[d] | | | 97.2 | | |

[a] After 29 h at 5 °C.
[b] After 6 h at room temperature.
[c] After 64 days at −20 °C.
[d] After 38 days at −20 °C.

## 4. Conclusions

A rapid, sensitive, and simple method for determining finasteride levels in human plasma was developed and validated. The UPLC–MS/MS method described herein boasts significant advantages over other techniques, including LC–MS/MS, due to the inherently increased column efficiency of UPLC, which resulted in complete analysis within 3.5 min with significantly lower limits of quantitation (0.1 ng/mL). This fully validated method can be an ideal tool for high-throughput analyses of clinical samples that are a critical component of pharmacokinetic studies and other clinical trials.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2010.04.029.

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