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Highly sensitive method for the determination of tamsulosin hydrochloride in human plasma dialysate, plasma and urine by high-performance liquid chromatography–electrospray tandem mass spectrometry

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Abstract

A highly sensitive method for the determination of tamsulosin hydrochloride, a structurally new type of sulphamoile derivative, in human plasma dialysate, plasma and urine has been developed by using liquid chromatography–electrospray tandem mass spectrometry (LC–MS–MS). Plasma dialysate, plasma and urine samples were extracted by brief liquid-phase extraction and analyzed using an HPLC system coupled to a mass spectrometer via an electrospray ionization interface. Selected reaction monitoring was used for the detection of tamsulosin and its internal standard. This method was validated in the concentration range 10–1000 pg/ml in plasma dialysate, 0.5–50 ng/ml in plasma, and 1–100 ng/ml in urine with sufficient specificity, accuracy and precision. The in vivo protein binding study demonstrated that the unbound tamsulosin in human plasma obtained by the equilibrium dialysis after 0.4-mg oral dosing was measurable. In addition, the percentage of unbound tamsulosin in an in vitro study (0.71–0.91%) obtained by using spiked ^{14}C-labelled tamsulosin was slightly larger than that of the in vivo study (0.68–0.86%), indicating that the unbound concentration calculated by the product of the plasma concentration and the in vitro unbound fraction (fu) was unfavorably overestimated. These results suggest that the combination of LC–MS–MS and equilibrium dialysis method has enough sensitivity to determine the unbound concentration in clinical use and gives the concentration more exactly than the in vitro fu.

Keywords: Tamsulin hydrochloride

1. Introduction

Tamsulosin hydrochloride, (−)-(*R*)-5-[2-[[2-(*o*-ethoxyphenoxy)ethyl]amino]propyl] - 2 - methoxybenzenesulfonamide hydrochloride, is a structurally new type of sulphamoile derivative, possessing a highly selective α_1-adrenoceptor antagonistic property [1,2]. An in vitro study revealed that the selectivity of this drug to prostate α_1-adrenoceptor was about 10 times higher than that to aorta α_1-adrenoceptor [3]. Tamsulosin has been used clinically for urinary obstructed patients with benign prostatic hyperplasia. In our recent study, it was revealed that tamsulosin was highly bound to α_1-acid glycoprotein (α_1-AGP) in plasma and the fraction bound to plasma protein varied with plasma α_1-AGP levels. Moreover, sub-

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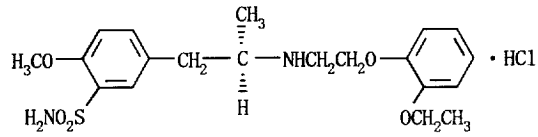
jects with higher plasma α_1-AGP levels tended to have high plasma concentrations of tamsulosin. Such an increase has been reported in the case of propranolol [4,5]. In these cases, the total plasma concentration is not an appropriate index of expected pharmacological effects or development of side effects, because such activities are a function of the unbound fraction of the drug [5–7]. It is important, therefore, to evaluate the unbound fraction of drugs in plasma and to monitor the unbound concentrations [8,9]. Although we have already reported a method for the determination of tamsulosin by high-performance liquid chromatography with fluorescence detection (HPLC–FL) [10], higher sensitivity is required for the determination of unbound tamsulosin in plasma which is not detectable by the former method. High-performance liquid chromatography–mass spectrometry (LC–MS) is a suitable technique for the sensitive and fast quantitation of some biological and xenobiotic compounds [11–13]. Thus, we have developed a high-performance liquid chromatography–electrospray tandem mass spectrometry (LC–MS–MS) method for the determination of unbound tamsulosin in plasma. Moreover, we have applied the LC–MS–MS method to the determination of tamsulosin in plasma and urine, enabling much faster pre-treatment than the previous method.

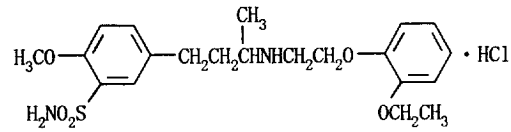
2. Experimental

2.1. Chemicals

Tamsulosin hydrochloride (I) and internal standard (II), (±)-(*R*)-5-[3-[[2-(*o*-ethoxyphenoxy) ethyl]amino]butyl] - 2 - methoxybenzenesulfonamide hydrochloride (AB289), were supplied by Yamanouchi Institute for Drug Discovery Research Laboratories. Their chemical structures are shown in Fig. 1. [^{14}C]Tamsulosin hydrochloride (specific activity 3.6 MBq/mg, radiochemical purity 99% or higher) was synthesized at Amersham International plc (Buckinghamshire, UK) and used for the study after purification by normal-phase preparative column chromatography. Sodium bicarbonate, potassium dihydrogen-phosphate, di-sodium hydrogenphosphate, ammonium acetate and methanol were purchased from Nacalai Tesque (Kyoto, Japan).



(I)



(II)

Fig. 1. Chemical structures of tamsulosin hydrochloride (I) and internal standard, AB289 (II).

Ethyl acetate, hexane and 2 *M* HCl were purchased from Kanto Chemical (Tokyo, Japan). These chemicals were all analytical grade.

2.2. Preparation of blank plasma dialysate

Blank plasma dialysate was prepared as follows: 100 ml of human blank plasma was transferred into dialysis tube (Spectra/Por®, MWCO 12–14 kd, Spectrum, Houston, TX, USA) that had been knotted at one end. Subsequently, the tube was knotted at the other open end, and then dialyzed against an equal volume of phosphate buffered isotonic solution (66 m*M* sodium phosphate in 50 m*M* NaCl adjusted to pH 7.4) with gentle stirring for 4 h at room temperature. The resultant buffer was used as blank plasma dialysate and stored at 4°C prior to sample preparation.

2.3. Chromatographic conditions

The HPLC system consisted of an LC-10AS pump, an SIL-10A autoinjector, a CTO-10 column oven and an SIL-10A controller (Shimadzu, Kyoto, Japan). Reversed-phase HPLC was performed by a J'sphere ODS-80H column (75 mm×4.6 mm I.D., 5 μm, YMC, Tokyo, Japan). The mobile phase consisted of 50 m*M* acetic acid adjusted to pH 4.0 with 50 m*M* ammonium acetate and methanol (4:6 v/v). The column temperature was maintained at 40°C and

a constant flow-rate of 0.5 ml/min was employed throughout the analyses.

2.4. Mass spectrometric conditions

A TSQ70 triple quadrupole mass spectrometer equipped with an API source (Finnigan MAT, San Jose, CA, USA) was used. The API source was fitted with an ESI inlet for ionizing the analytes in the HPLC eluent. Electrospray ionization was effected by a spray voltage of +4.5 kV. Heated capillary temperature was maintained at 270°C. High purity nitrogen served both as sheath gas with an operating pressure of 65 psi and as auxiliary gas with a flow-rate of 15 units. (The flow meter was not calibrated. Fifteen units corresponds approximately to 1.5–3 l/min.) Selected reaction monitoring (SRM) was used for the detection of I and II with a dwell time of 500 ms. The first quadrupole (Q1) was set up to transmit the molecular ions MH^+ at m/z 409 (I) and m/z 423 (II). These molecular ions were fragmented by collision activated dissociation with argon (1.2 mtorr) at −30 eV in the second quadrupole (Q2). The product ions were monitored in the third quadrupole (Q3) at m/z 228 and 285 for I and II, respectively.

2.5. Standard solutions

Stock solutions of I and II were prepared at 1 mg/ml in methanol and stored at −20°C. These solutions were stable for at least 18 months. The stock solution of I was further diluted with 0.05 *M* HCl to give a series of working solutions with concentrations of 0.1, 0.2, 0.5, 1, 2, 5 and 10 ng/ml for plasma dialysate assay, and 1, 2, 5, 10, 20, 50 and 100 ng/ml for plasma and urine assay. Working solutions of II were prepared by dilution of the stock solution with 0.05 *M* HCl at a concentration of 5 ng/ml for plasma dialysate assay and 100 ng/ml for plasma and urine assays.

2.6. Validation and quality control (QC) sample preparation

A stock solution of I for QC samples was prepared at 1 mg/ml in methanol and stored at −20°C. This solution was diluted with 0.05 *M* HCl to give a series of working solutions with concentrations of 0.3, 4, and 8 ng/ml for plasma dialysate QC samples, 50 and 1000 ng/ml for plasma QC samples, and 100, 1000, and 2000 ng/ml for urine QC samples. A 0.9-ml aliquot of blank plasma dialysate was pipetted into a 10-ml centrifuge tube and 100 μl of each working solution for plasma dialysate QC samples was spiked to make final concentrations of 30, 400 and 800 pg/ml. A 0.2-, 0.3- or 0.4-ml aliquot of the working solution for plasma or urine QC samples was diluted with blank plasma or blank urine and adjusted to the final volume of 10 ml with concentrations of 1.5, 20 and 40 ng/ml for plasma QC samples, and 3, 40 and 80 ng/ml for urine QC samples. A 0.2-ml aliquot of plasma QC samples and a 0.1-ml aliquot of urine QC samples were pipetted into a 10-ml centrifuge tube. Those QC samples were stored at −20°C prior to analysis.

2.7. Sample preparation

2.7.1. Plasma dialysate

To a 1-ml aliquot of plasma dialysate in a 10-ml centrifuge tube, 100 μl of internal standard solution (5 ng/ml), 1 ml of saturated sodium bicarbonate solution and 5 ml of ethyl acetate were added. The centrifuge tube was shaken for 10 min and was centrifuged for 5 min at 1200 *g*. The organic layer was removed into another centrifuge tube, and then was evaporated to dryness under reduced pressure. The residue was reconstituted in 100 μl of the mobile phase, and a 50-μl aliquot was injected onto the LC–MS–MS system.

2.7.2. Plasma and urine

To a 0.2-ml aliquot of plasma or a 0.1-ml aliquot of urine in a 10-ml centrifuge tube, 100 μl of internal standard solution (100 ng/ml), 1 ml of saturated sodium bicarbonate solution and 5 ml of hexane–ethyl acetate mixture (7:3, v/v) were added. The centrifuge tube was shaken for 10 min and centrifuged for 5 min at 1200 *g*. The organic layer was removed into another centrifuge tube, and then was evaporated to dryness under reduced pressure. The residue was reconstituted in 200 μl of the mobile phase, and a 50-μl aliquot was injected onto the LC–MS–MS system.

2.8. Accuracy and precision

Accuracy and precision of the LC–MS–MS method were determined by analyses of the QC samples with three different concentrations and the blank matrix spiked with the concentration of the expected lower limit of quantitation (LLOQ) for plasma dialysate, plasma and urine assays. Six replicates of each concentration were analyzed on one day. Inter-day accuracy and precision were assessed by analysing six replicates of the same QC samples with three different concentrations for plasma assay. This procedure was repeated on different days ($n=18$). The linearity of each standard curve was confirmed by plotting the peak-area ratio of I to II versus the nominal concentration of I. The calibration curves were obtained by weighted ($1/x^2$) linear regression analysis.

2.9. Protein binding study

2.9.1. In vivo and in vitro protein binding

Plasma samples which were obtained from a male volunteer receiving 0.4 mg of tamsulosin hydrochloride as a sustained-release formulation were used. Plasma unbound fraction of tamsulosin was obtained by equilibrium dialysis. One ml of the plasma was dialyzed with an equal volume of phosphate buffered isotonic solution (pH 7.4) for 4 h at 37°C in an equilibrium dialyzer (Spectrum) with 2×1.3 ml Teflon half cells separated by a cellulose membrane (Spectra/Por® 4 Discs, MWCO 12–14 kd). The concentrations of tamsulosin in the buffer and the plasma after dialysis were determined by LC–MS–MS to examine the in vivo protein binding. In addition, to determine whether results from in vitro protein binding measurements are consistent with those from in vivo measurements, in vitro bound or unbound fraction was measured in the plasma containing tamsulosin by using spiked ^{14}C-labelled tamsulosin and compared with the corresponding values of in vivo protein binding. To a 1-ml aliquot of the plasma, a 50-μl aliquot of [^{14}C]tamsulosin solution (phosphate buffered isotonic solution pH 7.4) was added to give a rather high concentration of 200 ng/ml to achieve the reliable determination, and then was dialyzed with an equal volume of the phosphate buffered isotonic solution as described above. One ml of the dialysate fluid and 0.2 ml of plasma after dialysis were used for the measurement of unbound and total [^{14}C]tamsulosin concentrations. These samples were mixed with 10 ml of scintillation cocktail (Aquasol-2, New England Nuclear, Boston, MA, USA), and then radioactivity was measured by a liquid scintillation counter (2000CA, Packard Instruments, Meriden, CT, USA). Percentage bound and unbound of tamsulosin were calculated.

2.9.2. Plasma tamsulosin concentration before and after dialysis

To examine whether the tamsulosin concentration in the plasma dialysate can be used as the plasma unbound concentration, [^{14}C]tamsulosin concentrations in plasma before and after dialysis were determined. [^{14}C]Tamsulosin was individually added to blank plasma which was obtained from 5 healthy male volunteers. For each plasma sample, triplicate determinations were carried out.

3. Results

3.1. Chromatography and mass spectrometry

The positive product ion mass spectra of the molecular ions of I (m/z 409) and II (m/z 423) are shown in Fig. 2. The most intensive product ion was observed at m/z 228 for I and m/z 285 for II, resulting from different fragmentation patterns. By monitoring these product ions fragmented from the molecular ions at m/z 409→228 for I and m/z 423→285 for II in the SRM mode, a highly sensitive assay for I was developed. The representative chromatograms of plasma dialysate, plasma and urine extracts analyzed by the LC–MS–MS method are shown in Fig. 3 Fig. 4 Fig. 5. No interfering peak was observed at the retention time of I (c.a. 2 min). Good linearity was observed over the concentration range 10–1000 pg/ml for plasma dialysate, 0.5–50 ng/ml for plasma, and 1–100 ng/ml for urine. The equations for the calibration curves were $y=0.0053x+0.0011$ ($r^2=0.997$), $y=0.0493x+0.0001$ ($r^2=0.998$), and $y=0.0231x+0.0052$ ($r^2=0.997$), respectively.

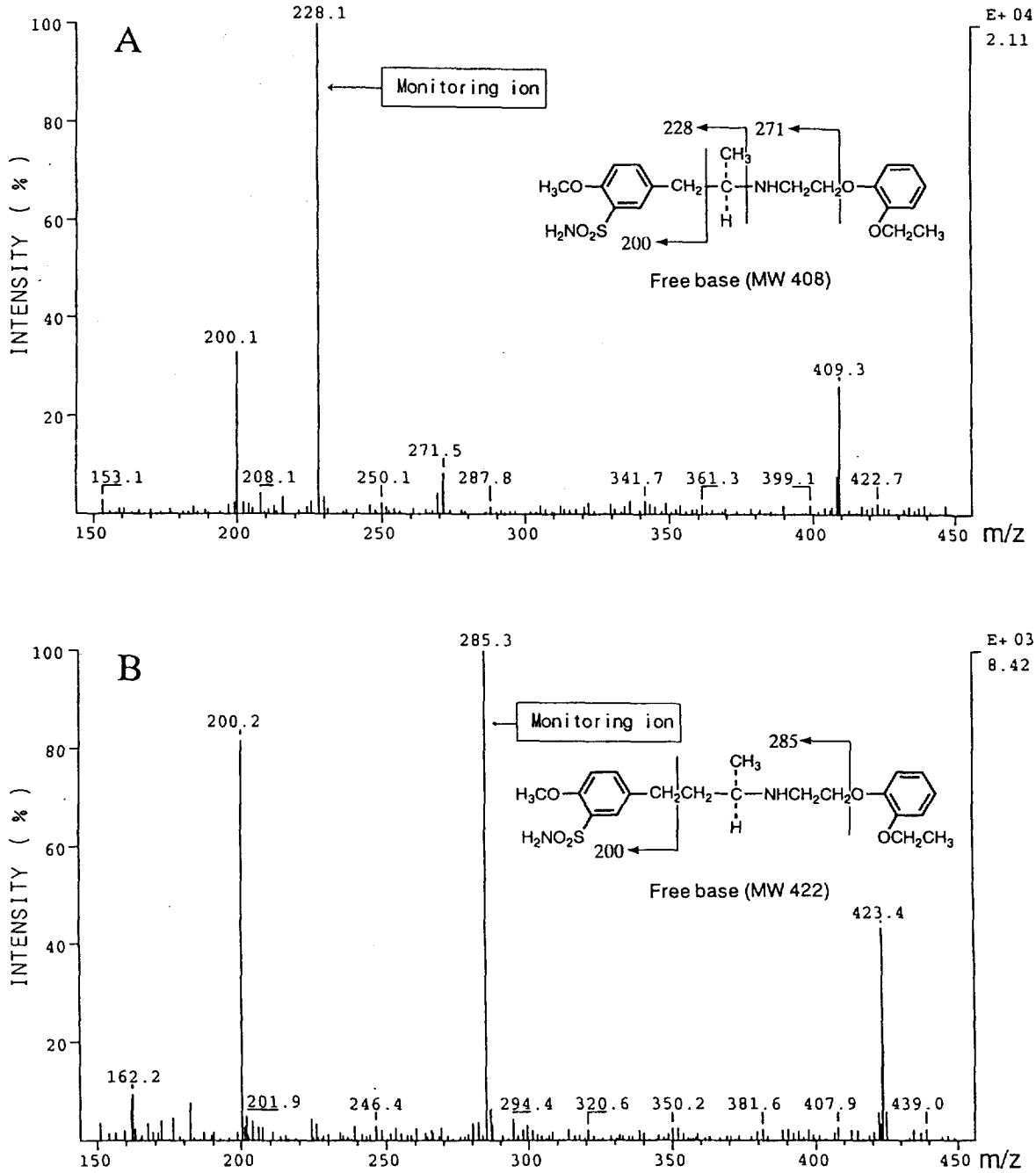


Fig. 2. Positive production mass spectra of the protonated molecular ions (MH^+) of I (m/z 409, A) and II (m/z 43, B).

3.2. Accuracy and precision

The intra- and inter-day accuracy and precision data are shown in Table 1. The intra-day precision expressed as coefficient of variance (C.V.) for each QC concentration was within 14.2% for plasma dialysate assay, within 8.7% for plasma assay, and within 9.6% for urine assay. The C.V.s at the LLOQ

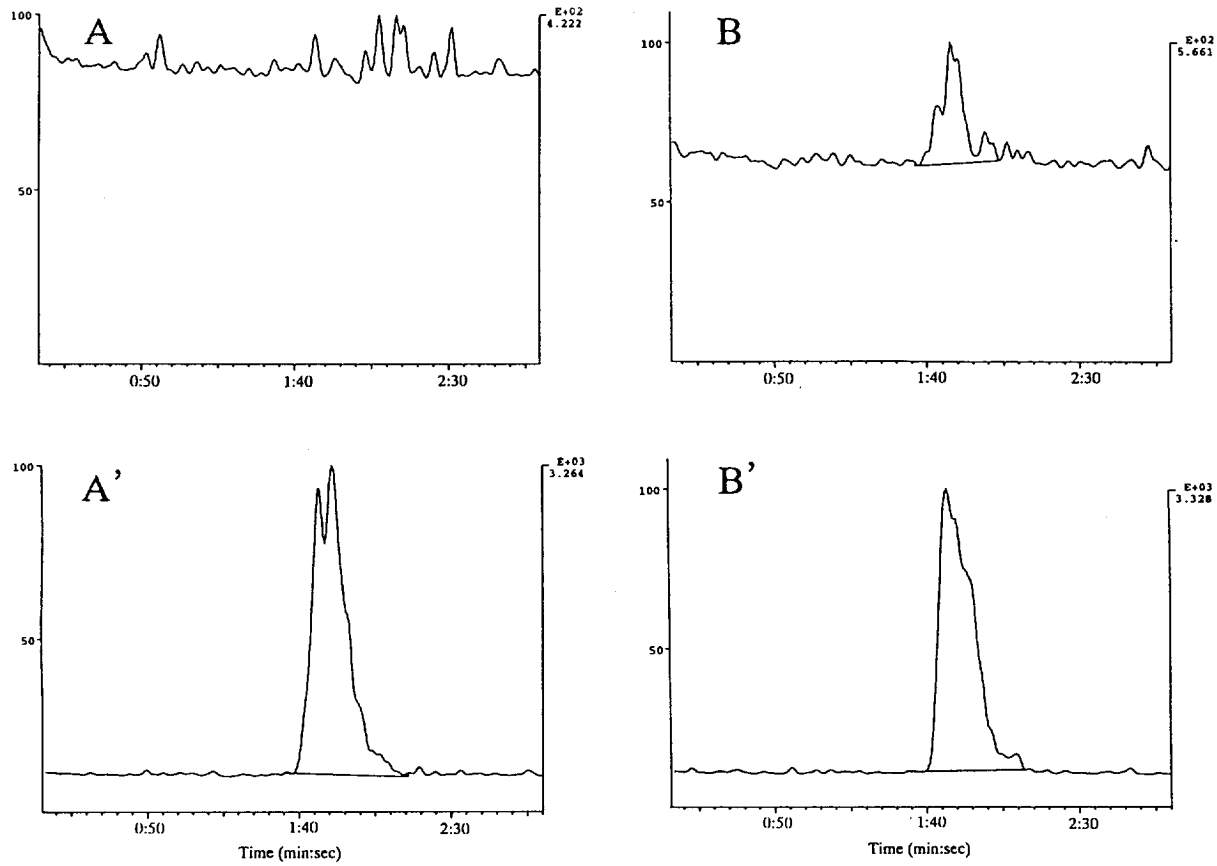


Fig. 3. Representative LC–MS–MS chromatograms of plasma dialysate extracts obtained by selected reaction monitoring at m/z 409→228 for I (A, B) and m/z 423→285 for II (A′, B′). A, A′: Blank plasma dialysate spiked with 0.5 ng of II. B, B′: Blank plasma dialysate spiked with 10 pg of I (LLOQ) and 0.5 ng of II.

were 17.7%, 13.3% and 18.9%, respectively. The intra-day accuracy (expressed as mean observed concentration/nominal concentration) for each QC concentration except for the LLOQ ranged 96.6–97.3% for plasma dialysate assay, 105.6–107.1% for plasma assay, and 109.6–109.9% for urine assay, and the accuracy for the LLOQ was 110.6%, 90.9% and 94.5%, respectively. The inter-day accuracy and precision for each QC concentration were in the range 102.6–105.9% and 3.3–6.7%, respectively, for plasma assay. Tamsulosin could be quantified with high sensitivity over the range 10–1000 pg/ml in plasma dialysate, and could be determined over the range 0.5–50 ng/ml in plasma and 1–100 ng/ml in urine. The extraction recovery of tamsulosin from these specimens was more than 80%. Tamsulosin is stable for at least 1 year in plasma and urine and for at least 3 months in plasma dialysate stored at −20°C.

3.3. Protein binding study

The results of in vivo and in vitro protein binding study are shown in Table 2. The in vivo % unbound of tamsulosin was 0.68–0.86%, slightly smaller than that observed in vitro (0.71–0.91%). The concentration of tamsulosin in the dialysate fluid was 141–192 pg/ml at 3 to 6 h after 0.4-mg oral dosing.

The results of [^{14}C]tamsulosin concentrations in plasma before and after dialysis are shown in Table 3. Although % unbound of [^{14}C]tamsulosin varied from 0.54% to 1.70%, plasma concentrations of

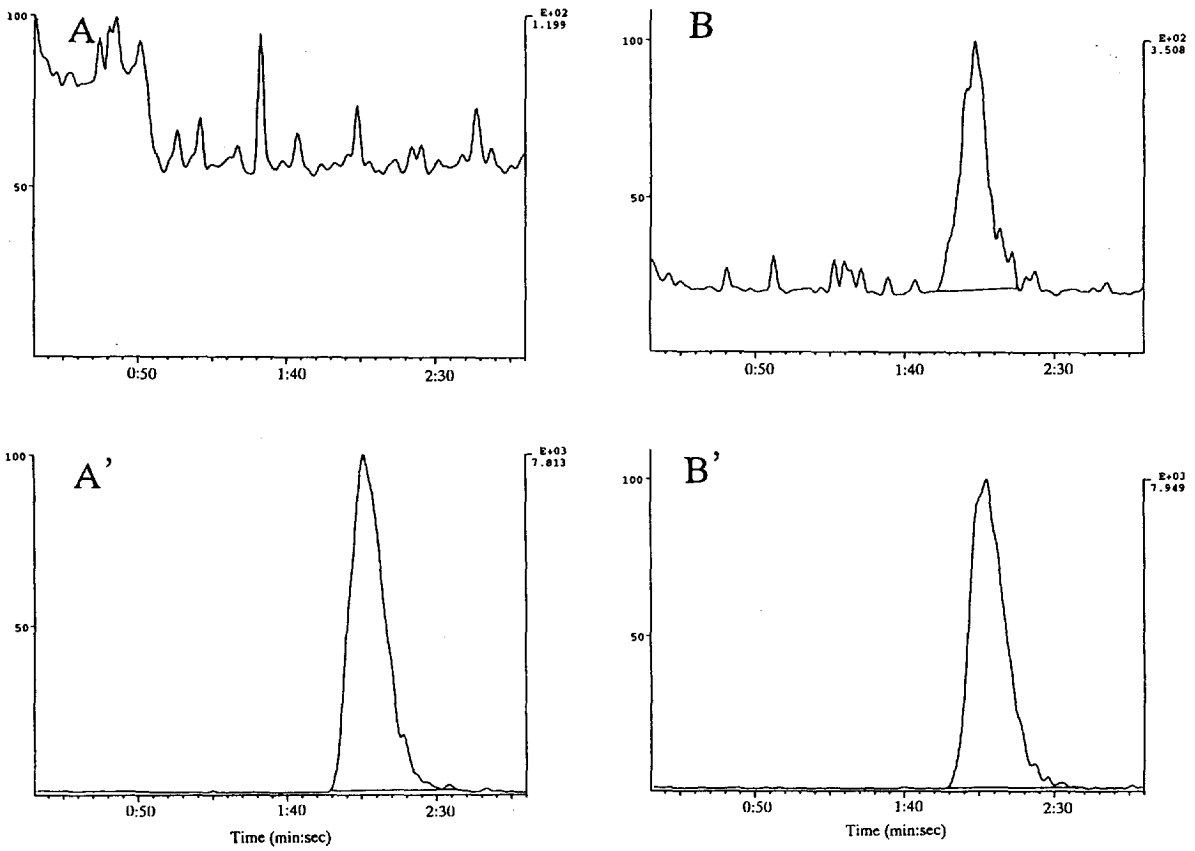


Fig. 4. Representative LC–MS–MS chromatograms of plasma extracts obtained by selected reaction monitoring at m/z 409→228 for I (A, B) and m/z 423→285 for II (A′, B′). A, A′: Blank plasma spiked with 10 ng of II. B, B′: Blank plasma spiked with 0.5 ng of I (LLOQ) and 10 ng of II.

[^{14}C]tamsulosin were not changed before and after dialysis.

4. Discussion

The results of our experiments indicate that high sensitivity can be achieved by using LC–MS–MS techniques. The analytical method developed for the determination of tamsulosin in human plasma dialysate has enough sensitivity and simplicity to measure the unbound drug concentration in human plasma. The intra-day accuracy and precision data ensured that tamsulosin could be quantified with high sensitivity with a lower limit of quantification of 10 pg/ml in the dialysate fluid. We have also applied this analytical method to the determination of tamsulosin in human plasma and urine. Although the HPLC–FL method which we had previously developed has enough sensitivity for the determination of tamsulosin in plasma, the LC–MS–MS method makes it possible to not only reduce the sample volume used for the assay, but also to simplify the pre-treatment procedures without lowering the sensitivity. This method, moreover, requires no separation of the compounds by HPLC and has a short analytical time of 3 min, giving more advantages than the previous method.

The higher sensitivity is attributed to the SRM detection, which can detect the selected fragment ion from its molecular ion [MH^+] with selectivity and specificity. The positive ion mass spectra of [MH^+] of tamsulosin (m/z 409) and the internal standard (m/z 423) indicated the presence of intense product

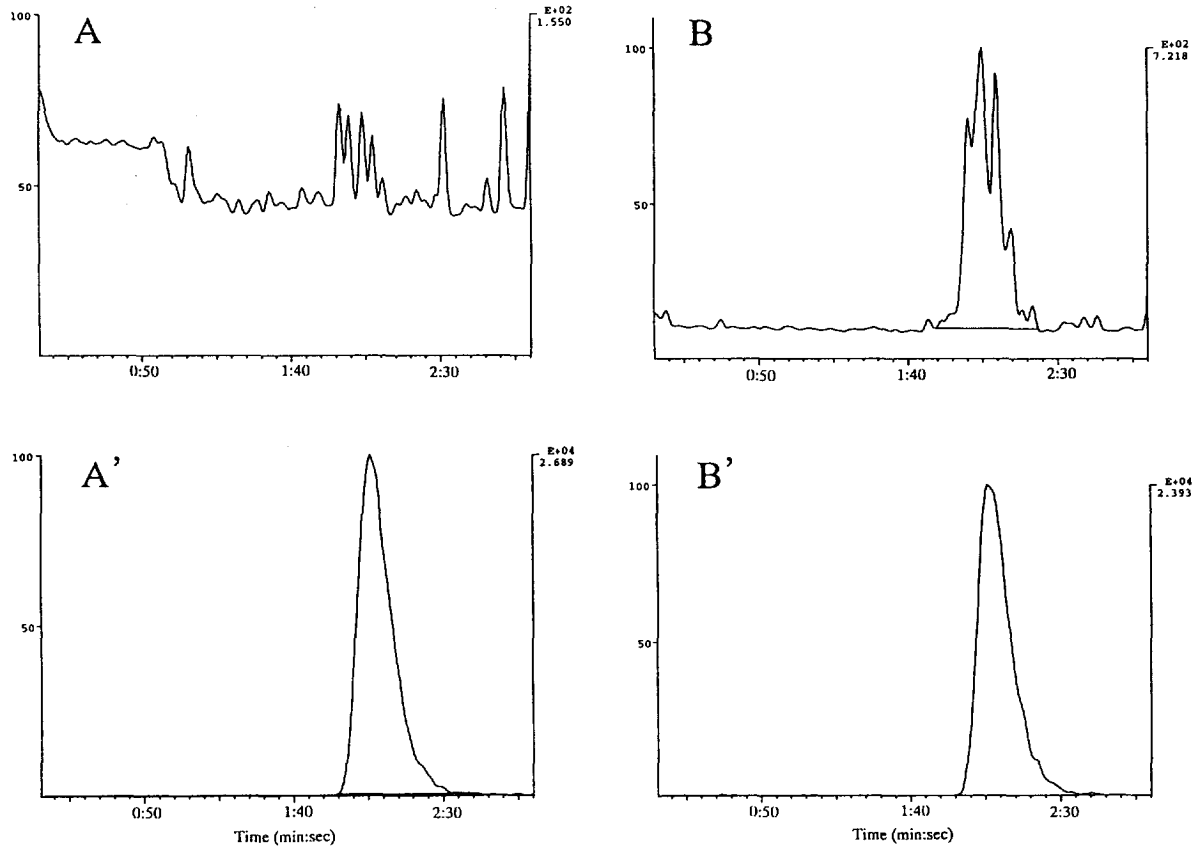


Fig. 5. Representative LC–MS–MS chromatograms of urine extracts obtained by selected reaction monitoring at m/z 409→228 for I (A, B) and m/z 423→285 for II (A′, B′). A, A′: Blank urine spiked with 10 ng of II. B, B′: Blank plasma spiked with 1 ng of I (LLOQ) and 10 ng of II.

ions at m/z 228, which represented the loss of the 2-(*o*-ethoxyphenoxy)ethyl amine moiety, and at m/z 285, which represented the loss of the *o*-ethoxyphenol moiety. These fragment ions are used as the targets of SRM detection in the analytical method. It is interesting that the fragment of the internal standard at m/z 242, which would be analogous fragment of tamsulosin at m/z 228, was not formed at all. This result suggests that the C–N bond in the internal standard is much more stable than that in tamsulosin. Collision energy must be set to the most appropriate conditions of SRM detection, since ion fragmentation of a parent ion is affected by the collision energy. In our preliminary study, the intensity of fragment ion of tamsulosin at m/z 228 decreased, whereas that at m/z 271 formed by the loss of the *o*-ethoxyphenol moiety slightly increased when the collision energy was reduced to −25 eV. In addition, the intensity of fragment ion at m/z 228 decreased, whereas that at m/z 200, which represented 2-methoxy-5-methyl benzene-sulfonamide moiety, increased when the collision energy was increased to −35 eV. These findings led us to conclude that the highest intensity of the target fragment was obtained at the collision energy of c.a. −30 eV.

Tamsulosin is oxidatively metabolized in rat, dog and human (Fig. 6) [14]. In addition, fragmentation observed in its 4 metabolites, which were *o*-deethylated (M-1), 4-hydroxylated (M-2), 5-hydroxylated (M-3) and *o*-demethylated (M-4) metabolites, was analogous to that observed in tamsulosin by

Table 1
Accuracy and precision of the LC–MS–MS method for the determination of tamsulosin in human plasma dialysate, plasma and urine

Matrix	Nominal concentration	n	C.V. (%)	Accuracy (%)
Plasma dialysate (pg/ml)	*Intra-day*			
	10[a]	6	17.7	110.6
	30	6	12.0	97.3
	400	6	14.2	96.8
	800	6	5.0	96.6
Plasma (ng/ml)	*Intra-day*			
	0.5[a]	6	13.3	90.9
	1.5	6	8.7	105.6
	20	6	2.4	107.1
	40	6	3.0	105.6
	Inter-day			
	1.5	18	6.7	102.6
	20	18	4.5	103.6
	40	18	3.3	105.9
Urine (ng/ml)	*Intra-day*			
	1[a]	6	18.9	94.5
	3	6	9.6	109.9
	40	6	3.6	109.6
	80	6	3.9	109.6

C.V.: Coefficient of variation.
[a] Concentration of the lower limit of quantitation (LLOQ).

LC–MS–MS detection. In our preliminary study, it was suggested that this LC–MS–MS technique was applicable to the determination of these metabolites.

In the in vitro binding study, % bound of tamsulosin was more than 98%, suggesting that tamsulosin is extensively bound to plasma protein and that the plasma tamsulosin concentrations differ little before and after dialysis. In fact, plasma concentrations of [^{14}C]tamsulosin were not changed before and after dialysis (Table 3). This result enables us to evaluate that the concentration in the dialysate fluid was equal to the unbound concentration in the plasma.

Yacobi et al. reported that % unbound of [^{14}C]warfarin, an extensively protein bound drug, was overestimated by degradation products or impurities [15]. This data implies that impurities of radiolabelled compound lead to overestimating the unbound plasma concentration, which is calculated by using the total plasma concentration after dosing and the in vitro unbound fraction (fu) obtained by determination of plasma spiked with the radiolabelled compound. Our in vitro studies, thereby, were performed by using the purified [^{14}C]tamsulosin to avoid overestimation due to degradation products. Purity check and/or purification of [^{14}C]tamsulosin, however, is tedious and inconvenient. In addition, the in vitro % unbound of [^{14}C]tamsulosin slightly increased compared to the in vivo % unbound. This slight increase seemed to be caused by the slight dilution of the plasma with [^{14}C]tamsulosin solution and/or by the increase in occupancy of the drug binding sites on the proteins due to the excess addition of the drug. For these reasons, the in vivo fu is more appropriate and reliable than the in vitro fu obtained by using the radiolabelled compound to estimate the unbound concentration of tamsulosin when it is given clinically in spite of small difference between the former and the latter. Moreover, the

Table 2
In vivo and in vitro protein binding of tamsulosin in plasma obtained after oral 0.4-mg dosing in the clinical study

Time after dosing (h)	Plasma concentration (ng/ml)	Unbound concentration (pg/ml)	% Unbound		% Bound	
			in vivo	in vitro	in vivo	in vitro
3	20.8	141	0.68	0.71	99.32	99.29
4	25.6	182	0.71	0.75	99.29	99.25
5	22.4	192	0.86	0.91	99.14	99.09
6	23.7	171	0.72	0.80	99.28	99.20

In vivo protein binding was determined by tamsulosin concentrations in the dialysate and the plasma after dialysis. Unbound concentration was obtained by the determination of concentration in the dialysate fluid. In vitro protein binding was determined by [^{14}C]tamsulosin concentrations in the dialysate and the plasma after dialysis. [^{14}C]Tamsulosin was spiked to the plasma samples obtained in this study. % unbound=$C_u/C_p\times100$, % bound=$(C_p-C_u)/C_p\times100$; C_p: plasma tamsulosin concentration, C_u: tamsulosin concentration in dialysate fluid.

Table 3
Plasma tamsulosin concentrations before and after dialysis

Subject No.	Plasma concentration (ng/ml)			% Unbound	% Bound
	Before dialysis	After dialysis	% difference		
1	179±2	174±1	97.3±0.6	0.54±0.01	99.46±0.01
2	176±3	175±2	99.6±1.4	0.88±0.04	99.12±0.04
3	175±1	178±2	101.2±1.2	1.35±0.03	98.65±0.03
4	177±1	177±2	99.8±0.9	0.80±0.004	99.20±0.004
5	175±1	178±1	101.9±0.7	1.70±0.05	98.30±0.05

Values are expressed as the mean of triplicate determinations ±SD.
% difference=C_p (after dialysis)/C_p (before dialysis)×100, % unbound=C_u/C_p×100, % bound=$(C_p-C_u)/C_p$×100; C_p: plasma [^{14}C]tamsulosin concentration, C_u: [^{14}C]tamsulosin concentration in dialysate fluid.

unbound concentration of tamsulosin obtained by the measurement of that in the dialysate fluid was 141–192 pg/ml at 3 to 6 h after 0.4-mg oral dosing, suggesting that this LC–MS–MS method had enough sensitivity to determine the unbound plasma concentration of tamsulosin in clinical use.

As mentioned in the introduction, tamsulosin is highly bound to α_1-acid glycoprotein (α_1-AGP). Plasma α_1-AGP levels are known to increase in morbid states such as infection [16], inflammation [17], cancer [18], myocardial infarction [19], trauma [20] or renal failure [17], and are also known to increase with aging [21]. In these cases, the elevated plasma α_1-AGP levels varied largely [19,20], resulting in transient alteration of the plasma protein binding. Such alteration would cause changes in total and unbound plasma concentrations of tamsulosin at steady state. As to a highly bound drug like tamsulosin, the unbound plasma concentration is an important determinant to evaluate expected pharma-

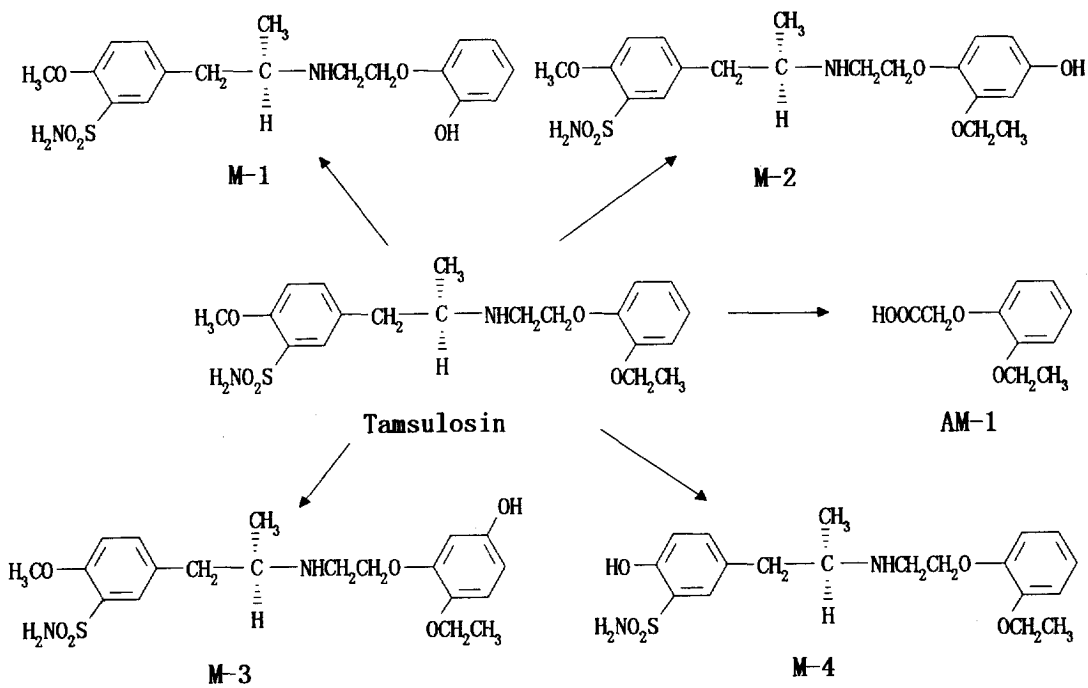


Fig. 6. Scheme for the oxidative metabolism of tamsulosin.

cological effects or development of adverse reactions. We have revealed in the present work that by using this LC–MS–MS method, the unbound tamsulosin concentrations can be measured and profiled conveniently after harvesting only 1 ml of plasma. Furthermore, effective and safe medications for the patients with benign prostatic hyperplasia, which is very common disease among aged men, should become possible by using this method.

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