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# Determination of atomoxetine in human plasma by a high performance liquid chromatographic method with ultraviolet detection using liquid–liquid extraction

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**Abstract**

A HPLC method with UV detection (210 nm) was developed and validated for the quantification of atomoxetine, a new medication for the treatment of attention deficit/hyperactivity disorder, in human plasma. Following a two-step liquid–liquid extraction with diethyl ether, the analyte and internal standard (maprotiline) were separated using an isocratic mobile phase of acetonitrile/phosphate buffer (39/61, v/v, pH 6.6) on a reverse phase Inertsil $C_{18}$ column. Linearity was verified over the range of 3.12–200 ng/mL atomoxetine in plasma. The lowest limit of detection is 2.5 ng/mL (S/N = 10). This HPLC method was validated with within- and between-batch precisions of 4.9–14.4% and 4.7–13.1%, respectively. The within- and between-batch biases were −1.9 to 1.4% and 0.1–13.8%, respectively. Commonly used psychotropic drugs and frequently coadministered drugs did not interfere with the drug and internal standard. This method is simple, economical and specific, and has been used successfully in a pharmacokinetic study of atomoxetine.


*Keywords:* Atomoxetine; Liquid–liquid extraction; HPLC; Pharmacokinetic

## 1. Introduction

Atomoxetine, a new therapeutic drug chemically known as (−)-*N*-methyl-3-phenyl-3-(*o*-tolyloxy)-propylamine hydrochloride (Fig. 1), was approved by the United States FDA in 2003 to treat attention deficit/hyperactivity disorder (ADHD) [1]. ADHD is a common neurobehavioral disorder marked by symptoms of inattention, hyperactivity and impulsiveness, which impair academic and social functioning. The disorder begins early in life, and the symptoms of some patients persist to adulthood. The prevalence of ADHD in children is 6–10% in the United States [2], 5–16% in Europe [3] and 3–13% in China [4,5].

Atomoxetine is the first approved non-stimulant drug for the treatment of ADHD. Prior to atomoxetine approval, most of children and adults diagnosed with ADHD were treated with psychostimulants such as methylphenidate, dexamfetamine, and pemoline, among others. Psychostimulants have been demonstrated to be effective in relieving the symptoms of ADHD [6,7]. These drugs exert their pharmacological effects via the mechanism of increasing availability of extracellular dopamine by blocking dopamine transporters and in some cases enhancing dopamine release [8]. The main problems associated with these medications are their potential for abuse, and failure to respond in some patients. Atomoxetine is a potent inhibitor of the presynaptic norepinephrine transporter, with minimal affinity for other noradrenergic receptors or for other neurotransporters or receptors [9,10]. It is not a stimulant and does not have the abuse potential associated with psychostimulants [11].

Several pharmacokinetic studies of atomoxetine with single and multiple doses in healthy volunteers [12–15], children and adolescents with ADHD [16], and ADHD patients with hepatic impairments [12] have been reported. Of note, most reports were from western populations. A systematic review of the pharmacokinetics of atomoxetine was recently published by Sauer et al. [17]. However, the pharmacokinetics of atomoxetine in different ethnic groups, the influence of co-medication on the concentration of atomoxetine, therapeutic drug mon-

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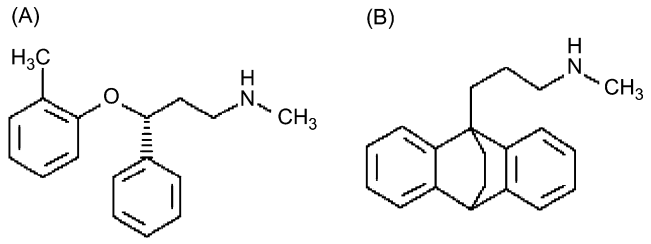


Fig. 1. Chemical structures of: (A) atomoxetine and (B) maprotiline (internal standard).

itoring, and other aspects of administration need further investigation.

So far, the reported analytical methods for atomoxetine include GC [15], LC–MS [18] HPLC-FL [19], and HPLC-UV [20]. The GC method reported by Farid et al. [15] for the quantification of atomoxetine in human plasma and urine used liquid–liquid extraction (LLE) and electron-capture detection with a structural analogue as internal standard. Recently, Mullen et al. [18] reported a liquid chromatography/tandem mass spectrometry (LC–MS/MS) method with atmospheric pressure chemical ionization (APCI) for the determination of atomoxetine and its metabolites (4-hydroxyatomoxetine, *N*-des-methylatomoxetine, and 4-hydroxyatomoxetine-*O*-glucuronide) in human plasma and urine, with a stable isotope labeled atomoxetine or/and 4-hydroxyatomoxetine as internal standard. Both methods have proved reliable for pharmacokinetic studies of atomoxetine in humans. However, the sample preparation for the GC method is laborious, and the LC–MS equipment is expensive and not widely available in clinical or bioassay research laboratories. Most recently, Zhu et al. [19] reported a liquid chromatography–fluorescence detection method for the quantification of atomoxetine in human plasma. The higher sensitivity and larger linear range made this method practical for pharmacokinetic study and therapeutic drug monitoring of atomoxetine. However, derivatization with the reagent 4-(4,5-diphenyl-1H-imidazol-2-yl) benzoyl chloride (DIB-Cl) during sample pretreatment is time-consuming and complex, thereby limiting applicability to routine clinical analysis. When the manuscript was under revising, Patel et al. [20] reported a HPLC-UV method using liquid–liquid extraction with tertiary butyl methyl ether. The lower limit of atomoxetine quantification was 50 ng/mL with the detection wavelength at 272 nm.

This paper describes a simple and selective HPLC-UV method without derivatization for the quantification of atomoxetine concentrations in human plasma with much lower limit of detection than the HPLC-UV method reported. Additionally, this paper also provides information regarding the stability of atomoxetine in different matrixes and conditions.

## 2. Experimental

### 2.1. Chemicals

Atomoxetine hydrochloride (99.6% pure) was obtained from Beijing Honglin Pharmaceutical Co. (Beijing, China). Maprotiline hydrochloride (internal standard, IS) was obtained from the Beijing Institute of Pharmacology and Toxicology (Beijing, China). Chemical structures are presented in Fig. 1. HPLC-grade acetonitrile was from Merck (Darmstadt, Germany). Diethyl ether was re-distillated before using. Purified de-ionized water from Milli-Q system (Millipore, Bedford, MA, USA) was used. Sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium phosphate and other chemicals were of analytical grade.

### 2.2. Chromatography

The integrated high performance liquid chromatography system (LC P1000, Thermo Electron Corporation, CA, USA) was equipped with an isocratic pump, an auto-sampler, an UV detector and a data system (ChromQuest version 4.1). The separation of compounds was made on an Inertsil ODS-3 column (5 μm, 150 mm × 4.6 mm i.d.) at room temperature. The column was preceded by a Phenomenex SecurityGuard$^{TM}$ $C_{18}$ guard column (4.0 mm × 3.0 mm i.d.).

The mobile phase consisted of acetonitrile and 23 mM phosphate buffer (pH 6.6) in the ratio of 39:61 (v/v), pumped at a flow-rate of 1.0 mL/min. Detection was set at the wavelength of 210 nm. Peak height ratios of the atomoxetine peak to the IS peak were employed for all calculations (performed in Microsoft Excel).

### 2.3. Sample collection

Venous blood samples were collected in single-use vacuum tubes (Insepack, China) with sodium heparin anticoagulant from 12 healthy male volunteers (19–25 years old, nonsmokers) before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12, 16 and 24 h post-atomoxetine dose. Plasma was separated within 1 h with centrifugation (1200 × *g* for 5 min at room temperature), and was stored at −70 °C until analysis. This protocol was approved by the Institutional Review Board at Beijing Anding Hospital, Capital Medical University, and a written informed consent was obtained from each subject.

For the preparation of in-house quality controls and calibration samples, drug-free human plasma containing sodium heparin anticoagulant was taken from other healthy volunteers.

### 2.4. Sample processing

A 1 mL plasma sample was transferred to a 10 mL glass test tube, and then 50 μL of IS working solution (1 μg/mL) and 500 μL 0.5 M PBS (pH 9.90) were added. The mixture was extracted twice with 2 mL portions of diethyl ether added using Dispensette Organic (Brand GmbH, Postfach, Germany). The sample was vortex-mixed for 4 min using a GL-88B Vortexer (QiLin Medical Products, Zhejiang, China). The sample was then centrifuged using BFX5-180 (Kangshou, Beijing, China) for 5 min at 1200 × *g*. The combined organic layer was transferred to a clean test tube containing 200 μL of 0.2 M HCl. After vortex-mixing for 3 min and centrifugation for 5 min, the organic layer was discarded. The acid solution was evaporated to dryness at above 90 °C under a stream of nitrogen. The dried extract was

then reconstituted with 120 μL of mobile phase, and an 80 μL was injected into the chromatographic system.

### 2.5. Calibration and quality control

Stock solutions of atomoxetine and of the internal standard were prepared separately in acetonitrile each at a concentration of 1 mg/mL and stored at −20 °C. Working solutions of atomoxetine for calibration (0.0625, 0.125, 0.25, 0.5, 1, 2 and 4 μg/mL), controls (0.16, 0.8 and 3.2 μg/mL) and LLOQ (0.0625 μg/mL) were prepared from the stock solutions by an adequate dilution using acetonitrile. The IS working solution (1 μg/mL) was also prepared by diluting stock solution with acetonitrile. Fifty microliters of working solution was evaporated to dryness at 40 °C under a stream of nitrogen added to 1 mL of drug-free plasma to obtain atomoxetine concentrations of 3.12, 6.25, 12.5, 25, 50, 100 and 200 ng/mL. Fifty-microliter working solutions of quality controls evaporated to dryness were added to 1 mL drug-free plasma in pool, to obtain atomoxetine concentrations of 3.12 ng/mL (LLOQ), 8 ng/mL (low), 40 ng/mL (medium) and 160 ng/mL (high), as a single batch at each concentration. The quality control pools were divided into aliquots of 1 mL in Eppendorf tubes (1.5 mL) and stored in the freezer at −70 °C until analysis.

Each validation run consisted of a blank sample (drug free plasma sample), seven non-zero calibration samples covering the total range (3.12–200 ng/mL), and QC samples at three concentrations ($n = 2$, at each concentration). Calibration samples were analyzed from low to high at the beginning of each validation run and other samples were distributed randomly through the run. Linearity was assessed by a weighted ($1/x^2$) least squares regression analysis. A correlation coefficient ($r$) of 0.99 or better was mandated for the calibration curve. The acceptance criterion for each back-calculated standard concentration was 15% deviation from the nominal value, except LOQ, which was set at 20%. At least 67% of non-zero standards were required to meet the above criteria.

## 3. Results

### 3.1. Separation

Fig. 2 shows the representative chromatograms of blank plasma, plasma sample spiked with 3.12 ng/mL (LLOQ) of atomoxetine and IS, plasma sample spiked with 100 ng/mL of atomoxetine and IS, and plasma sample obtained from a healthy volunteer 0.5 h after administration of an oral 20 mg dose of atomoxetine. The analyte separated well from IS under the described chromatographic conditions, at retention times of 5.2 and 6.5 min, respectively. No interference with constituents from the plasma matrix was observed.

### 3.2. Linearity, sensitivity and detection limit of the assay

The peak height ratio of atomoxetine to IS in human plasma was linear with respect to the analyte concentration over the range 3.12–200 ng/mL. The best fit for the calibration curve could be achieved with the linear equation $y = bx + a$ with a $1/x^2$ weighing factor. The mean linear regression equation of calibration curves for the analyte was $y = 0.0141\ (\pm 0.0007)\ x + 0.0082$

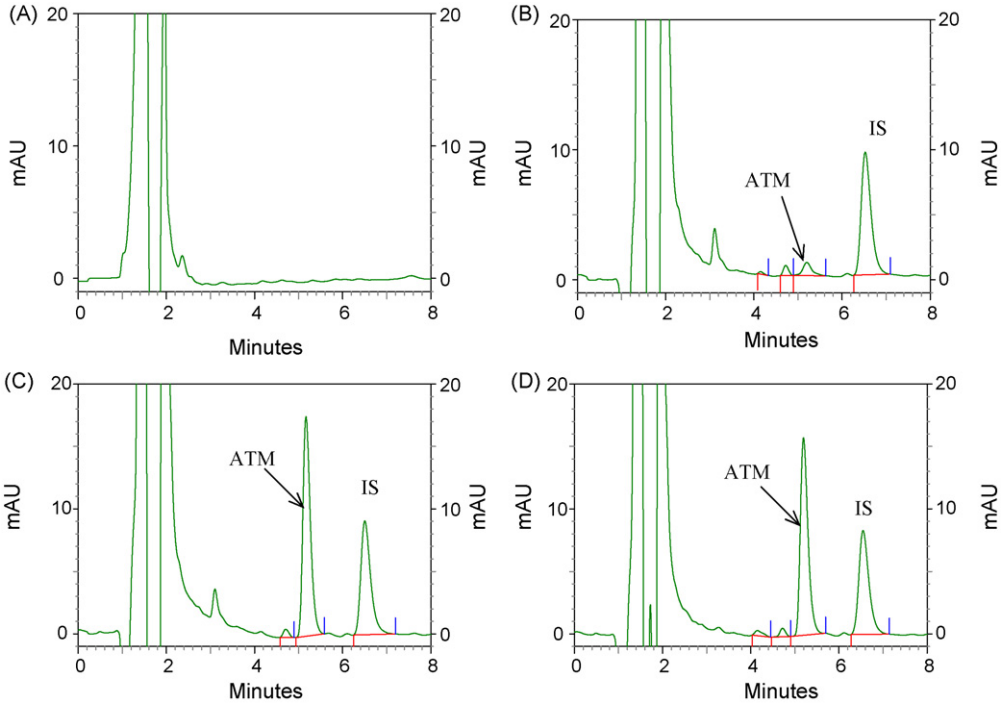


Fig. 2. Chromatograms of: (A) blank human plasma; (B) human plasma sample spiked with 3.12 ng/mL of atomoxetine (ATM) and IS; (C) human plasma sample spiked with 100 ng/mL of atomoxetine and IS; (D) plasma sample from a healthy volunteer 0.5 h after administration of a 20 mg oral dose of atomoxetine. The plasma concentration was determined to be 115 ng/mL. Approximate retention times: atomoxetine = 5.2 min; IS = 6.5 min.

Table 1
Precision and accuracy of atomoxetine calibration curve points in human plasma ($n = 7$)

| Added concentration (ng/mL) | Measured concentration (mean ± S.D., ng/mL) | Accuracy (%) | Precision (R.S.D.) |
|---|---|---|---|
| 3.12 | 3.14 ± 0.18 | 0.5 | 5.9 |
| 6.25 | 6.39 ± 0.77 | 2.2 | 12.1 |
| 12.5 | 12.0 ± 0.8 | −3.9 | 6.3 |
| 25 | 23.1 ± 2.2 | −7.5 | 9.7 |
| 50 | 50.8 ± 2.3 | 1.6 | 4.5 |
| 100 | 103 ± 5 | 3.9 | 5.3 |
| 200 | 201 ± 22 | 0.6 | 11.1 |

(±0.0087) ($n = 7$), where $y$ was the peak height ratio of the analyte to the IS and $x$ was the concentration of the analyte in plasma. The correlation coefficient ($r$) for atomoxetine was above 0.99 over the concentration range used. Table 1 summarizes the calibration curve results for the analyte. These calibration curves were suitable for generation of acceptable data for the concentrations of the analyte in the calibration samples during method validation and subject sample detection.

The limit of detection, defined as the concentration of atomoxetine that produces a signal-to-noise ratio of 10, was 2.5 ng/mL.

### 3.3. Extraction recovery

The extraction recovery of atomoxetine at 8, 40 and 160 ng/mL as well as IS at 50 ng/mL was evaluated by comparing the peak heights of six quality control samples subjected to extraction to the mean peak heights of three unprocessed reference solutions, respectively.

The extraction recovery of atomoxetine at low, medium and high quality control samples was 71.4 ± 5.7%, 73.8 ± 2.7% and 72.4 ± 3.8%, respectively. This indicates that extraction recovery of atomoxetine is independent of concentration. The recovery of IS was 77.3% at the concentration used in the assay.

### 3.4. Specificity

Six randomly selected blank human plasma samples, collected from different healthy volunteers, were carried through the extraction procedure and chromatographed to determine whether endogenous plasma components interfere with the analyte or internal standard. We did not find interfering peaks present at the retention time of either the analyte or IS.

Since most of children with ADHD also have comorbid psychiatric disorders, it was important to demonstrate that atomoxetine and IS were free of interference from other potentially co-administered drugs, such as antidepressants, antipsychotics, sedative-hypnotics and psychostimulants. Every drug standard solution was diluted to about 20 μg/mL by mobile phase, 2–5 μL of the solution was injected for analysis, separately. Table 2 lists compounds that do not interfere with the analysis.

### 3.5. Accuracy and precision of the assay

Within-batch accuracy and precision evaluations were performed by analysis of samples consisting of calibration samples and six replicates of LLOQ, low, medium and high quality control samples for atomoxetine. Between-batch accuracy and precision were assessed by repeated analysis of atomoxetine on three separate batches. The overall precision of the method is expressed as relative standard deviation, and accuracy of the method is expressed in terms of bias.

The accuracy values for between- and within-batch studies at LLOQ, low, medium and high concentrations of atomoxetine in human plasma were within acceptable limits ($n = 6$) (Table 3). The results also indicated that the assay method was reproducible for replicate analysis of atomoxetine within the same batch and on different batches.

### 3.6. Stability

(A) Freeze-thaw stability of the samples was obtained over triplicate freeze-thaw cycles, by thawing at room temperature for 2–3 h and refreezing at −70 °C for 12–24 h for each cycle.
(B) Twenty-four hour stability was examined by keeping replicates at room temperature for approximately 24 h.
(C) Autosampler stability of atomoxetine was tested by analysis of processed and reconstituted samples, which were stored in the autosampler tray for 24 h.
(D) Stability of atomoxetine in human plasma was tested after storage at −70 °C for 30 days.
(E) Samples were freshly prepared and processed immediately. For each concentration and each storage condition, three low and high concentration replicates were analyzed in one analytical batch.

Each storage period solution was considered stable if analyte peak height after extraction was within or equal to ±7% of the freshly prepared solution peak height.

Table 2
Drugs tested that do not interfere with atomoxetine analysis

| Therapeutic class | Drug names |
|---|---|
| Antidepressants | Paroxetine, fluoxetine, reboxetine, venlafaxine, citalopram, sertraline, trazotone, trimipramine, clomipramine, doxepin, nordoxepin, norclomipramine, amitriptyline, desimipramine |
| Antipsychotics | Risperidone, 9-OH risperidone, olanzapine, quetiapine, clozapine, norclozapine, sulpiride, perphenazine, chlorpromazine, haloperidol, reduced haloperidol |
| Sedative-hypnotics | Zaleplon, diazepam, nitrazepam, estazolam, alprazolam |
| Psychostimulants | Methylphenidate |

Table 3
The accuracy, within- and between-batch precision and recovery data for the measurement of atomoxetine in human plasma

| Added concentration (ng/mL) | Within-batch ($n=6$) | | | Between-batch ($n=3$) | | |
|---|---|---|---|---|---|---|
| | Concentration found (mean ± S.D.) (ng/mL) | Precision (%) | Accuracy (%) | Concentration found (mean ± S.D.) (ng/mL) | Precision (%) | Accuracy (%) |
| 3.12 | 3.07 ± 0.39 | 14.4 | −1.9 | 3.56 ± 0.44 | 13.1 | 13.8 |
| 8 | 7.99 ± 0.74 | 9.2 | −0.1 | 8.01 ± 0.97 | 12.1 | 0.1 |
| 40 | 40.6 ± 2.2 | 5.3 | 1.4 | 41.3 ± 1.9 | 4.7 | 3.1 |
| 160 | 160 ± 8 | 4.9 | −0.1 | 171 ± 11 | 6.6 | 7.0 |

All stability results are summarized in Table 4. These results are in agreement with atomoxetine stability in plasma as previously reported by Mullen and co-workers [18].

### 3.7. Dilution accuracy

An evaluation of dilution accuracy was conducted to assess whether the upper concentration limit (200 ng/mL) could be extended. Quality control samples ($n=6$) at concentration 300 ng/mL were diluted once with the same volume of blank plasma, and the assay accuracy was determined in a similar manner as described in Section 2.5. For atomoxetine, the measured concentration was 328 ± 10 ng/mL with bias of 9.3%. The results suggest that samples whose concentrations are greater than the upper limit of the standard curve can be re-analyzed with appropriate dilution.

### 3.8. Application to clinical study

The present HPLC-UV method was employed to determine the atomoxetine levels in Chinese volunteers' plasma samples. After a single oral dose of 20 mg atomoxetine capsule (Beijing Honglin Pharmaceutical Co., Beijing, China) in 12 healthy volunteers, concentration versus time profiles were constructed for up to 24 h for atomoxetine quantification (Fig. 3). The maximum atomoxetine plasma concentration of volunteers was 183 ± 58 ng/mL, and $t_{1/2}$ was 3.27 ± 0.80 h. The parameter values supported the results reported previously [15]. All 12 individuals' pharmacokinetic profiles showed double peaks or multiple peaks. This phenomenon will be investigated in a later study.

## 4. Discussion

A validated HPLC-UV method for the quantification of atomoxetine in human plasma is reported here. As ultraviolet absorption data of atomoxetine is scant, we investigated the peak heights of atomoxetine with the same amount of injection at different wavelengths from 190 to 380 nm. We found that the absorption intensity from 230 to 380 nm was not strong enough to develop a sensitive analytical method with UV detection; within that range, the wavelengths for maximum UV absorption occurred at 271 and 380 nm. We also found that the peak height of atomoxetine at 210 nm is 12.7 times higher than that at 271 nm and 7.8 times higher than that at 380 nm. When the wavelength is less than 210 nm, the absorption intensity strengthens, but higher baseline noise and endogenous interferences were unavoidable. The detection of atomoxetine at 210 nm provided maximum intensity with minimum interference.

Table 4
Stability of atomoxetine at different conditions

| Stability | Peak height of low concentration (8 ng/mL) | Ratio (%) | Peak height of high concentration (160 ng/mL) | Ratio (%) |
|---|---|---|---|---|
| (E) Concentration of fresh preparation ($n=3$) | 1104 | 100 | 20701 | 100 |
| (A) Three freeze and thaw cycles ($n=3$) | 1100 | 99.6 | 20910 | 101.0 |
| (B) Stability for 24 h ($n=3$) | 1043 | 94.5 | 20867 | 100.8 |
| (C) Autosampler stability for 24 h ($n=3$) | 1036 | 93.8 | 20126 | 97.2 |
| (D) 30-days stability at −70 °C ($n=3$) | 1052 | 95.3 | 19824 | 95.8 |

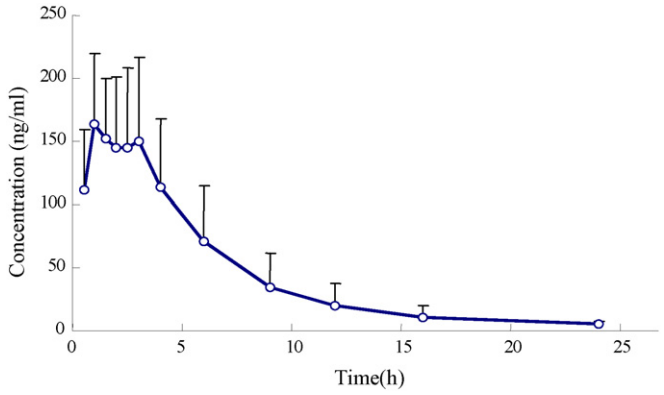


Fig. 3. Mean concentrations vs. time profile over 24 h of atomoxetine in human plasma from 12 healthy volunteers receiving a 20 mg single oral dose of atomoxetine.

Both solid phase extraction (SPE) and liquid–liquid extraction (LLE) sample preparation methods have been reported [15,18–20]. The SPE method used polystyrenedivinylbenzene to extract atomoxetine from plasma and urine [18]. For routine clinical analysis, a high-throughput method with SPE carries higher cost than LLE. Both Farid et al. [15] and Zhu et al. [19] reported LLE methods with derivatization, which made these methods complex. In order to develop a simple liquid–liquid extraction procedure with sufficient and stable recovery, we developed a two-step LLE method without derivatization.

For an internal standard, we selected maprotiline, which exhibits chromatographic and extraction properties similar to those of atomoxetine. Maprotiline is a commercially available antidepressant and is seldom co-administered with atomoxetine for the treatment of ADHD.

The linear range of an analytical method should cover the analyte concentration in the samples. The reported literature shows that the average maximum plasma concentration ($C_{max}$) of atomoxetine in extensive metabolisers is 142 ng/mL after a 20 mg single oral dose [12], and 160–184 ng/mL after oral administration of 20 mg twice daily [13,14]. The half life of atomoxetine in human plasma of extensive metabolisers is 3.1–5.3 h [17]. In poor metabolisers, the $C_{max,ss}$ is six times higher than that in extensive metabolisers, and the half life is about 14.1–26.8 h after multiple dosing [21]. It seems that a larger linear range is needed for the detection of atomoxetine in samples of poor metabolisers. But, as a matter of fact, atomoxetine is predominantly metabolised by CYP2D6 [22], and the incidence of CYP2D6 poor metabolisers among Chinese is only 1% [23]. Our work has already demonstrated that a linear range from 3.12 to 200 ng/mL for the assay herein could meet the requirements for a pharmacokinetic study of atomoxetine after a single oral dose of 20 mg. The subjects of this experiment are highly likely to be all extensive metabolisers. The dilution accuracy data demonstrate that atomoxetine plasma samples with higher concentration could be detected by dilution with blank plasma. These facts conceivably warrant the reliable extension of this method to the pharmacokinetic study of atomoxetine in poor metabolisers.

We have demonstrated that plasma internal substances, potentially co-administered drugs and other substances do not interfere atomoxetine and IS, but the selectivity for atomoxetine with its two major metabolites, 4-hydroxyatomoxetine and *N*-desmethylatomoxetine, have not been proofed, because of a lack of availability of the standards. According to the paper of Sauer et al. [21], the influence of the CYP2D6 polymorphism on the overall disposition and metabolism of a 20 mg dose of $^{14}$C-atomoxetine was evaluated in CYP2D6 extensive metabolisers ($n = 4$) and poor metabolisers ($n = 3$) under steady-state conditions. In the plasma of CYP2D6 extensive metabolisers, 4-hydroxyatomoxetine and *N*-desmethylatomoxetine plasma levels are only 1.3% and 4.4% of the atomoxetine concentration, respectively. That means both 4-hydroxyatomoxetine and *N*-desmethylatomoxetine would yield very little interference to the detection of atomoxetine in CYP2D6 extensive metabolisers, even if these two metabolites are not separated from atomoxetine. In CYP2D6 poor metabolisers, 4-hydroxyatomoxetine was undetectable, but *N*-desmethylatomoxetine level was much higher (approximately 28.3% of the atomoxetine concentration) and would gave a strong interfere to the detection of atomoxetine. Seven percent of the Caucasian population and 1% of the Asian population are poor metabolisers of CYP2D6 substrates [23]. This is a problem which could not be neglected when this method is used in TDM practice. Nevertheless, we do not think this is a big problem for analyzing atomoxetine. We have also compared the chromatograms of the plasma samples before and after 20 mg single dose of atomoxetine from each 1 of the 12 individuals. We found that there was a peak ($R_t$: 4.15–4.25 min; peak height: 180–410) in some chromatograms, which could not be seen in the chromatograms of the samples before administration. As the concentration of 4-hydroxyatomoxetine is very low, we think that this may be the peak of *N*-desmethylatomoxetine. However, this has not been verified at this point.

In conclusion, the developed HPLC-UV method shows good precision, accuracy and high extraction efficiency, with less expense and labor than the current methodologies. While the sensitivity of this method does not compare to the previously published HPLC methods with mass spectrometry or fluorescence detection, it is well-suited by way of simplicity and reliability to be applied in pharmacokinetic studies and possibly in therapeutic drug monitoring of atomoxetine.

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## References

[1] FDA, Consumer 37 (2003) 4.

[2] American Academy of Pediatrics, Committee on Quality Improvement and Subcommittee on Attention-Deficit/Hyperactivity Disorder, Pediatrics 105 (2000) 1158.
[3] K.N. Dwivedi, R.G. Banhatti, Arch. Dis. Child. 90 (2005) i10.
[4] Y.Z. Hu, H.R. Wu, J.G. Yu, Chin. J. School Doctor 5 (1998) 321.
[5] L. Lu, Q.J. Shi, F.F. Tao, P. Yin, S.X. Yin, Chin. Mental Health J. 4 (2006) 221.
[6] R.A. Barkley, J. Clin. Psychiatry 63 (2002) 36.
[7] A.R. Jadad, M. Boyle, C. Cunningham, M. Kim, R. Schachar, Evid. Rep. Technol. Assess. (Summ.) 11 (1999) 1.
[8] B. Giros, M.G. Caron, Trends Pharmacol. Sci. 14 (1993) 43.
[9] D.T. Wong, P.G. Threlkeld, K.L. Best, F.P. Bymaster, J. Pharmacol. Exp. Ther. 222 (1982) 61.
[10] D.R. Gehlert, D.A. Schober, S.L. Gackenheimer, J. Neurochem. 64 (1995) 2792.
[11] S.H. Heil, H.W. Holms, W.K. Bickel, S.T. Higgins, G.J. Badger, H.F. Laws, D.E. Faries, Drug Alcohol Depend. 67 (2002) 149.
[12] S.A. Chalon, J.P. Desager, K.A. DeSante, R.F. Frye, J. Witcher, A.J. Long, J.M. Sauer, J.L. Golnez, B.P. Smith, H.R. Thomasson, Y. Horsmans, Clin. Pharmacol. Ther. 73 (2003) 178.
[13] D.J. Belle, C.S. Ernest, J.M. Sauer, B.P. Smith, H.R. Thomasson, J.W. Witcher, J. Clin. Pharmacol. 42 (2002) 1219.
[14] J.M. Sauer, A.J. Long, B. Ring, N.P. Sanburn, K.A. DeSante, D. Petullo, M.R. VandenBranden, C.B. Jensen, S.A. Wrighton, B.P. Smith, H.A. Read, J.W. Witcher, J. Pharmacol. Exp. Ther. 308 (2004) 410.
[15] N.A. Farid, R.F. Bergstrom, E.A. Ziege, C.J. Parli, L. Lemberger, J. Clin. Pharmacol. 25 (1985) 296.
[16] J.W. Witcher, A.J. Long, J.M. Sauer, J. Heilgenstein, T. Wilens, T. Spencer, J. Biederman, J. Child. Adolesc. Psychopharmacol. 13 (2003) 53.
[17] J.M. Sauer, B.J. Ring, J.W. Witcher, Clin. Pharmacokinet. 44 (2005) 571.
[18] J.H. Mullen, R.L. Shugert, G.D. Ponsler, Q. Li, B. Sundaram, H.L. Coales, J.E. Yakupkovic, R.M. Lelacheur, W.J. Wheeler, F.J. Belas, J.M. Sauer, J. Pharm. Biomed. Anal. 38 (2005) 720.
[19] H.J. Zhu, J.S. Wang, J.L. Donovan, C.L. Devane, B.B. Gibson, J.S. Markowitz, J. Chromatogr. B 846 (2006) 351.
[20] C. Patel, M. Patel, S. Rani, M. Nivsarkar, H. Padh, J. Chromatogr. B 850 (2007) 356.
[21] J.M. Sauer, G.D. Ponsler, E.L. Mattiuz, A.J. Long, J.W. Witcher, H.R. Thomasson, K.A. Desante, Drug. Metab. Dispos. 31 (2003) 98.
[22] B.J. Ring, J. Gillespie, J.A. Eckstein, S.A. Wrighton, Drug. Metab. Dispos. 30 (2002) 319.
[23] L. Bertilsson, Clin. Pharmacokinet. 29 (1995) 192.