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Determination of atomoxetine metabolites in human plasma by liquid chromatography/tandem mass spectrometry and its application to a pharmacokinetic study

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ABSTRACT

4-Hydroxyatomoxetine (4-HAT) and *N*-desmethylatomoxetine (N-DAT) are major metabolites of atomoxetine, a potent and selective inhibitor of the presynaptic norepinephrine transporter that is used for the treatment of attention deficit/hyperactivity disorder. The pharmacological activity of 4-HAT is similar to that of atomoxetine. We have developed and validated a simple, rapid and sensitive liquid chromatography analytical method with tandem mass spectrometry (LC–MS/MS) for the determination of 4-HAT and N-DAT in human plasma. After liquid–liquid extraction with methyl *t*-butyl ether, chromatographic separation of analytes was performed using a reversed-phase Luna C_{18} column (2.0 mm × 100 mm, 3 μm particles) with a mobile phase of 10 mM ammonium formate buffer (pH 3.5)–methanol (10:90, v/v) and quantified by MS/MS detection in ESI positive ion mode. The flow rate of the mobile phase was 250 μL/min and the retention times of 4-HAT, N-DAT and internal standard (IS, metoprolol) were 0.9, 1.0 and 1.0 min, respectively. The calibration curves were linear over the range of 0.05–20 ng/mL for 4-HAT and 0.1–20 ng/mL for N-DAT. The lower limits of quantification, using 200 μL human plasma, were 0.05 and 0.1 ng/mL for 4-HAT and N-DAT, respectively. The mean accuracy and precision for intra- and inter-day validation of 4-HAT and N-DAT were both within the acceptable limits. This LC–MS/MS method showed improved sensitivity for quantification of the two main metabolites of atomoxetine in human plasma compared with previously described analytical methods. The validated method was successfully applied to a pharmacokinetic study in humans.

1. Introduction

Atomoxetine ((−)-*N*-methyl-3-phenyl-3-(o-tolyloxy)-propylamine), a potent and selective inhibitor of the presynaptic norepinephrine transporter that has minimal affinity for other monoamine transporters and receptors, causes an increase in levels of dopamine and norepinephrine in the prefrontal cortex [1–3]. Atomoxetine is approved for the treatment of attention-deficit/hyperactivity disorder (ADHD) in children, adolescents, and adults.

After an oral administration, atomoxetine is rapidly absorbed and primarily cleared from the body via three metabolic pathways: aromatic ring-hydroxylation, benzylic hydroxylation, and *N*-demethylation. Among these pathways, aromatic ring-hydroxylation, which yields 4-hydroxyatomoxetine (4-HAT) and is primarily mediated by the CYP2D6 enzyme, is the major metabolic pathway [4,5]. *N*-demethylation to *N*-desmethylatomoxetine (N-DAT), another metabolite of atomoxetine, is primarily mediated by CYP2C19 [5]. 4-HAT selectively blocks presynaptic norepinephrine transporters in a manner similar to atomoxetine; however, other oxidative metabolites are pharmacologically inactive relative to atomoxetine [6]. Therefore, it is important to determine the plasma concentrations of the major metabolites of atomoxetine, especially 4-HAT, as well as the parent drug.

Several analytical methods for the determination of atomoxetine using liquid chromatography coupled with ultraviolet detection [7,8], fluorescence [9], or tandem mass spectrometry (LC–MS/MS) [10,11] have been reported. These methods are useful for the pharmacokinetic study of atomoxetine. However, although methods for the determination of 4-HAT and N-DAT using an LC–MS/MS system have been reported [10,11], the lower limits of quantification (LLOQ) for both metabolites (1 ng/mL) do not meet

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requirements for the measurement of these compounds in human plasma. In healthy CYP2D6 extensive metabolizers, the maximum plasma concentration of 4-HAT and N-DAT after a 20 mg single oral dose of atomoxetine was only 1.92 ng/mL and 4.11 ng/mL, respectively [12]. Another study showed that the steady-state maximum plasma concentration of 4-HAT after 20 mg doses of atomoxetine administered twice daily over six days was 2.03 ng/mL in CYP2D6 extensive metabolizers. Furthermore, plasma concentrations of 4-HAT were not detectable in CYP2D6 poor metabolizers [6]. The requirement for large volumes of plasma (500 μL) and relatively long run times (5–20 min) are also obstacles to the successful determination of atomoxetine metabolites in human plasma [10,11].

In this study, we developed a new LC–MS/MS method for the simultaneous determination of the two metabolites of atomoxetine in human plasma using only 200 μL of human plasma and with LLOQ values of 0.05 ng/mL for 4-HAT and 0.1 ng/mL for N-DAT. This method was validated and evaluated through a pharmacokinetic study of the two metabolites of atomoxetine in humans.

2. Materials and methods

2.1. Reagents and chemicals

4-HAT and N-DAT were purchased from Toronto Research Chemical Inc. (North York, ON, Canada). Metoprolol was purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade methanol was purchased from J.T. Baker (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA). Ammonium formate was purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were analytical grade and were used without further purification.

2.2. Chromatographic instruments and conditions

Chromatography was performed on an Agilent 1200 series HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA). Separation was carried out on a 30 °C Luna C_{18} column (2.0 mm × 100 mm, 3 μm, Phenomenex Inc., Torrance, CA, USA). The mobile phase consisted of 10 mM ammonium formate buffer (pH 3.5) and methanol (10:90, v/v) at a flow rate of 250 μL/min. The autosampler was maintained at 4 °C. The total run time for each sample analysis was 2.0 min.

Mass spectrometric detection was performed on an API 3200 tandem mass spectrometer (Applied Biosystems/MDS SCIEX, Toronto, ON, Canada) equipped with an electrospray ionization (ESI) source. The mass spectrometer was operated in positive ion mode and the tandem mass spectrometry conditions for 4-HAT, N-DAT and internal standard (IS, metoprolol) were optimized by carrying out full scans in positive ion detection mode. The detection and quantification of 4-HAT, N-DAT and IS were performed in multiple reaction monitoring (MRM) modeQuadrupoles Q1 and Q3 were set to a unit resolution. Data acquisition and quantitation were carried out using Analyst software version 1.4.2 (Applied Biosystems/MDS SCIEX).

2.3. Standard solution and quality control sample preparation

Stock solutions of 0.1 mg/mL 4-HAT, N-DAT and metoprolol (IS) were prepared by dissolving these compounds in methanol. Standard working solutions (1, 5, 20, 100, 200 and 400 ng/mL for 4-HAT and 2, 10, 20, 100, 200 and 400 ng/mL for N-DAT) were prepared by diluting the stock solutions with 10 mM ammonium formate buffer (pH 3.0) and methanol (10:90, v/v). These working solutions were used for daily preparation of standard calibrators in human plasma at concentrations of 0.05, 0.25, 1, 5, 10 and 20 ng/mL for 4-HAT and 0.1, 0.5, 1, 5, 10 and 20 ng/mL for N-DAT. The quality control (QC) working solution was prepared in the same way as the standard working solutions. The QC samples at concentrations of 0.05, 0.15, 3 and 15 ng/mL for 4-HAT and 0.1, 0.3, 3 and 15 mg/mL for N-DAT, were prepared by diluting the working solution with blank human plasma. Medium (3 ng/mL) and high (15 ng/mL) concentrations were made by diluting the stock and working solutions with blank human plasma. The working IS solution (metoprolol 300 ng/mL) was prepared by diluting the stock solution with 10 mM ammonium formate buffer (pH 3.0) and methanol (10:90, v/v). All stock, standard working and QC working solutions were stored at −20 °C.

2.4. Sample preparation

The samples were stored in a freezer at −70 °C and allowed to thaw at room temperature before processing. Two hundred microliters of plasma sample and 10 μL of IS solution (metoprolol, 300 ng/mL) were added to a glass tube. After brief vortexing, 3 mL of methyl *t*-butyl ether (MTBE) was added and the mixture was vortexed for 60 s. After centrifugation at 3000 rpm for 10 min, the organic layer was transferred to a new glass tube and evaporated to dryness under a gentle stream of nitrogen gas at 50 °C. The residue was reconstituted with 300 μL 10 mM ammonium formate buffer (pH 3.0) and methanol (10:90, v/v) and a 10 μL aliquot was injected into the HPLC system.

2.5. Method validation

The validation was performed based on 'Guidance for Industry: Bioanalytical Method Validation' from the United States Food and Drug Administration (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf).

2.5.1. Selectivity and linearity

Selectivity was assessed by comparing the chromatograms of six different batches of plasma obtained from six subjects. The plasma samples were spiked with 4-HAT, N-DAT and IS. The linearity of the method was evaluated using five different calibration curves (ranges 0.05–20 ng/mL for 4-HAT and 0.1–20 ng/mL for N-DAT). The calibration curves were obtained by plotting the area ratios of each analyte and IS vs. the concentration of each analyte by least-squares linear regression with $1/x$ (where x represents the concentration of each analyte in ng/mL) as the weighing factor. The LLOQ was defined as the lowest concentration yielding a signal to noise ratio of at least 10 with a coefficient of variation (CV) < 20% and accuracy of 80–120%. The LLOQ was analyzed five times for confirmation.

2.5.2. Matrix effect and recovery

The matrix effect and recovery tests were performed in triplicate at three different QC sample concentrations (0.15, 3, and 15 ng/mL for 4-HAT and 0.3, 3, and 15 ng/mL for N-DAT). The matrix effect was determined by extracting blank human plasma from six different sources and then reconstituting the final extract in the injecting solvent, which contained known amounts of the analyte and IS. Absolute recovery of the analytes in normal plasma was determined by extraction from blank human plasma samples spiked with the analytes. After extraction, recovery was calculated by comparing the responses of plasma QC samples that were spiked with analytes prior to extraction with the response of those that were spiked with blank plasma.

2.5.3. Accuracy and precision

The intra-day and inter-day precisions were determined by replicate analysis of five sets of QC samples that were spiked with four different concentrations of analytes within one day or on five consecutive days. The precision was determined to be the CV

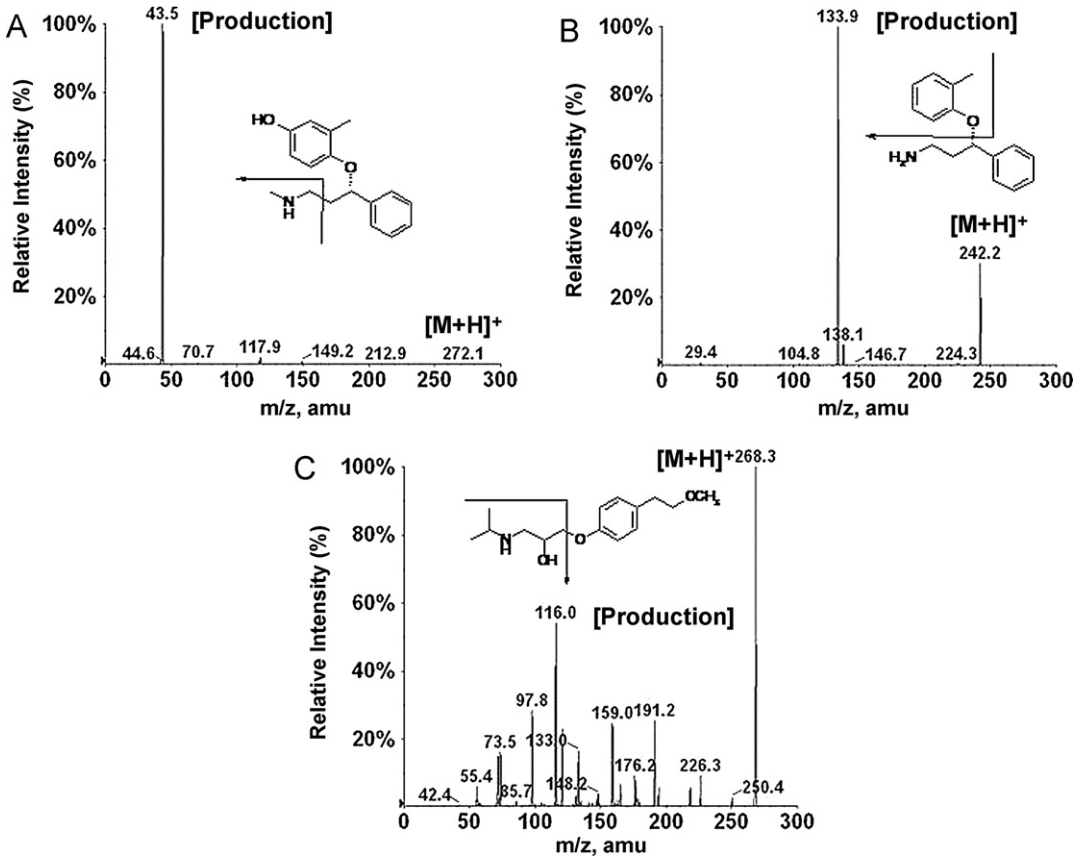

Fig. 1. Product ion mass spectra of (A) 4-hydroxyatomoxetine, (B) *N*-desmethylatomoxetine and (C) metoprolol (IS).

and the accuracy is expressed as the relative standard error (RSE (%) = measured concentration/targeted concentration × 100).

2.5.4. Stability

The stability of the analytes in human plasma was tested in triplicate with three different concentrations of QC sample (0.15, 3, and 15 ng/mL for 4-HAT and 0.3, 3, and 15 ng/mL for N-DAT). For short-term stability, frozen plasma samples (−70 °C) were kept at room temperature for 4 h before sample preparation. The freeze–thaw stability of the analytes was determined over three freeze–thaw cycles within three days. In each freeze–thaw cycle, the spiked plasma samples were frozen for 24 h at −70 °C and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 12–24 h at −70 °C. Long-term stability was evaluated after storing the frozen plasma samples at −70 °C for 60 days. The stability of the prepared plasma samples was tested after keeping the samples in the autosampler at 4 °C for 30 h. Stability samples were processed and extracted along with the freshly spiked calibration curve standards. The samples were considered stable if assay values were within the acceptable limits of accuracy (85–115%) and precision (±15%).

2.6. Pharmacokinetic application

Twelve Korean male volunteers were enrolled in this study. All subjects were healthy as defined by their medical histories, physical examinations, and routine laboratory tests (blood cell count, biochemical profile, and urinalysis). The subjects were restricted from ingesting any medications, caffeine, grapefruit products, or alcoholic beverages for at least one week before and during the study period. All subjects provided informed consent both verbally and in writing. The study was performed according to the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the School of Pharmacy at Sungkyunkwan University (Suwon, Korea).

After an overnight fast, on the day of the study each subject received one 40 mg oral dose of atomoxetine (Strattera®, Eli Lilly Korea Ltd., Seoul, Korea) with 240 mL water. The subjects were maintained in the fasting state for 4 h after the drug administration. Venous blood samples (7 mL) were obtained before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, and 24 h after the administration of atomoxetine. Blood samples were centrifuged immediately and the plasma fractions were stored at −70 °C until needed.

Pharmacokinetic parameters of 4-HAT and N-DAT were estimated using non-compartmental methods and BA Calc 2007 software provided by the Korean Food and Drug Administration (KFDA, Seoul, Korea). Actual blood sampling times were used and observed values were maximum plasma concentrations (C_{max}) and times to reach C_{max} (T_{max}). The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal rule. The elimination rate constant (k_e) was determined by linear regression analysis of the log-linear portion of the plasma concentration–time curve. The AUC from hour 0 to infinity (AUC_{inf}) was calculated as follows: $AUC_{inf} = AUC_t + C_t/k_e$ (where C_t is the last plasma concentration measured). The half-life ($t_{1/2}$) was calculated from the following equation: $\ln 2/k_e$.

3. Results and discussion

3.1. Method development

The tandem mass ESI conditions for 4-HAT, N-DAT, and IS were first optimized by carrying out full scans in positive ion detection mode. During a direct infusion experiment, the mass spectra for

Table 1
Precision and accuracy of the LC–MS/MS assay method for plasma 4-hydroxyatomoxetine and *N*-desmethylatomoxetine.

Targeted concentration (ng/mL)	Intra-day ($n=5$)			Inter-day ($n=5$)		
	Measured concentration (ng/mL)	Accuracy (%)	Precision (CV%)	Measured concentration (ng/mL)	Accuracy (%)	Precision (CV%)
4-Hydroxyatomoxetine						
0.05	0.054 ± 0.003	107.6	5.2	0.050 ± 0.004	99.3	7.4
0.15	0.149 ± 0.008	99.6	5.2	0.152 ± 0.007	101.3	4.5
3	2.96 ± 0.16	98.7	5.3	3.06 ± 0.07	102.1	2.4
15	14.6 ± 0.3	97.6	1.8	15.2 ± 0.6	101.3	4.0
N-Desmethylatomoxetine						
0.1	0.103 ± 0.008	103.0	7.5	0.099 ± 0.008	99.3	7.8
0.3	0.296 ± 0.015	98.8	5.1	0.296 ± 0.010	98.6	3.5
3	2.87 ± 0.10	95.8	3.4	2.91 ± 0.16	97.0	5.7
15	14.8 ± 0.4	98.5	2.7	14.4 ± 0.8	96.0	5.9

4-HAT, N-DAT, and IS showed peaks as protonated molecular ions $[M+H]^+$ at 272.0, 242.0, and 268.0 *m/z*, respectively. The major fragment ions observed in each product spectrum were at 44.0 *m/z* for 4-HAT, 134.0 for N-DAT and 116.0 for IS (Fig. 1). The mass parameters were optimized by observing the maximum responses of the product ions. The adjusted values of declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP) for 4-HAT were 25 V, 5 V, 40 V, and 3 V, respectively, and those for N-DAT were 24 V, 4 V, 10 V and 3 V. Optimized curtain gas (CUR), collision gas (CAD), nebulizer gas (GS1) and turbo gas (GS2) parameters were 35 psi, 6 psi, 70 psi, and 70 psi, respectively.

Chromatographic conditions, particularly the composition of the mobile phase, were optimized by several trials to increase the signal of the analytes and minimize running times. The mobile phase consisted of a mixture of 10 mM ammonium formate buffer (pH 3.5) and methanol. Ammonium formate is primarily used to improve the peak shape and promote source ionization. The optimal proportion of ammonium formate buffer and methanol was tested from 10:90 to 40:60 (v/v), and we selected a ratio of 10:90 (v/v) as the final condition of the mobile phase. The flow rate of the mobile phase was set to 250 μL/min. The chromatographic sensitivity was good using the Luna C_{18} column (2.0 mm × 100 mm, 3 μm).

3.2. Method validation

3.2.1. Selectivity and linearity

No endogenous interference was found at the retention times of 4-HAT, N-DAT and IS. Fig. 2 shows representative chromatograms for blank human plasma (Fig. 2A); human plasma spiked with 4-HAT (0.05 ng/mL), N-DAT (0.1 ng/mL), and IS (metoprolol 300 ng/mL; Fig. 2B); and a plasma sample obtained from a healthy volunteer at 6 h after oral administration of 40 mg atomoxetine (Fig. 2C). The LLOQ was 0.05 ng/mL for 4-HAT and 0.1 ng/mL for N-DAT, and the signal-to-noise ratio of LLOQ was >10. The intra- and inter-day CV was <8% for each analyte (Table 1). The standard calibration curves were linear over analyte concentration ranges in human plasma of 0.05–20 ng/mL for 4-HAT and 0.1–20 ng/mL for N-DAT with mean correlation coefficients of 0.9982 and 0.9975 for 4-HAT and N-DAT, respectively ($n=5$). The best linear fit and least-squares residuals for the calibration curve were achieved with a $1/x$ weight factor.

3.2.2. Recovery and matrix effect

Ethyl ether, MTBE, dichloromethane, and ethyl acetate were tested as solvents for extraction of 4-HAT and N-DAT from human plasma. Among these, MTBE was found to be the best solvents, producing a clean chromatogram for blank human plasma samples with the best recovery and least matrix effect. The recovery of analytes from a 200 μL plasma sample using the liquid–liquid extraction procedure with MTBE was measured at three different concentrations of QC sample. Regardless of drug concentration, mean recoveries for 4-HAT, N-DAT and IS were 70.0 ± 0.5%, 74.6 ± 0.8%, and 79.2 ± 1.9%, respectively.

Matrix effects, which are phenomena of ion suppression or enhancement of the analyte of interest, should be evaluated during method development because the assay accuracy and precision of the HPLC–MS/MS method are significantly affected [13,14]. Matrix effects on the analytes and IS were between 94.6% and 104.7%, and the CV values from six lots of plasma were less than 9.4%, indicating that no co-eluting substances influenced the ionization of either the analytes or the IS [15]. This result indicates that the extraction efficiency for analytes using liquid–liquid extraction was satisfactory, consistent, and concentration-independent. In addition, these results showed that ion suppression or enhancement from the plasma matrix was consistent under the current conditions.

3.2.3. Accuracy and precision

Table 1 provides a summary of the accuracy and precision for four concentrations of analytes (0.05, 0.15, 3 and 15 ng/mL for 4-HAT and 0.1, 0.3, 3, and 15 ng/mL for N-DAT). The intra-day and inter-day accuracies for 4-HAT and N-DAT were 97.3%–107.7% and 96.0%–103.2%, respectively. The intra- and inter-day precisions for 4-HAT and N-DAT were 1.8–7.5% and 2.4–7.8%, respectively. These results suggest that the method assessed in this study has satisfactory accuracy, precision, and reproducibility.

3.2.4. Stability

Stability was assessed under a variety of conditions and the results are summarized in Table 2. Three freeze–thaw cycles of the QC samples did not appear to affect the quantification of the analytes. The QC samples were stored in a freezer at −70 °C and remained stable for at least 60 days. Thawing the frozen samples and maintaining them at room temperature for 4 h had no effect on quantification. The extracted samples were also analyzed after at least 30 h at 4 °C. These results suggest that human plasma samples containing 4-HAT and N-DAT can be handled under normal laboratory conditions without any significant compound loss.

3.3. Incurred sample reanalysis

Incurred sample reanalysis (ISR) was performed for both 4-HAT and N-DAT. Of the 156 analyzed human plasma samples, 17 (11%) were chosen and reanalyzed to evaluate the reproducibility of the analytical method. The selection of samples covered the whole range of concentrations from the maximum plasma concentration (C_{max}) to the terminal elimination phase in the pharmacokinetic profile. The original concentrations of 4-HAT and N-DAT were

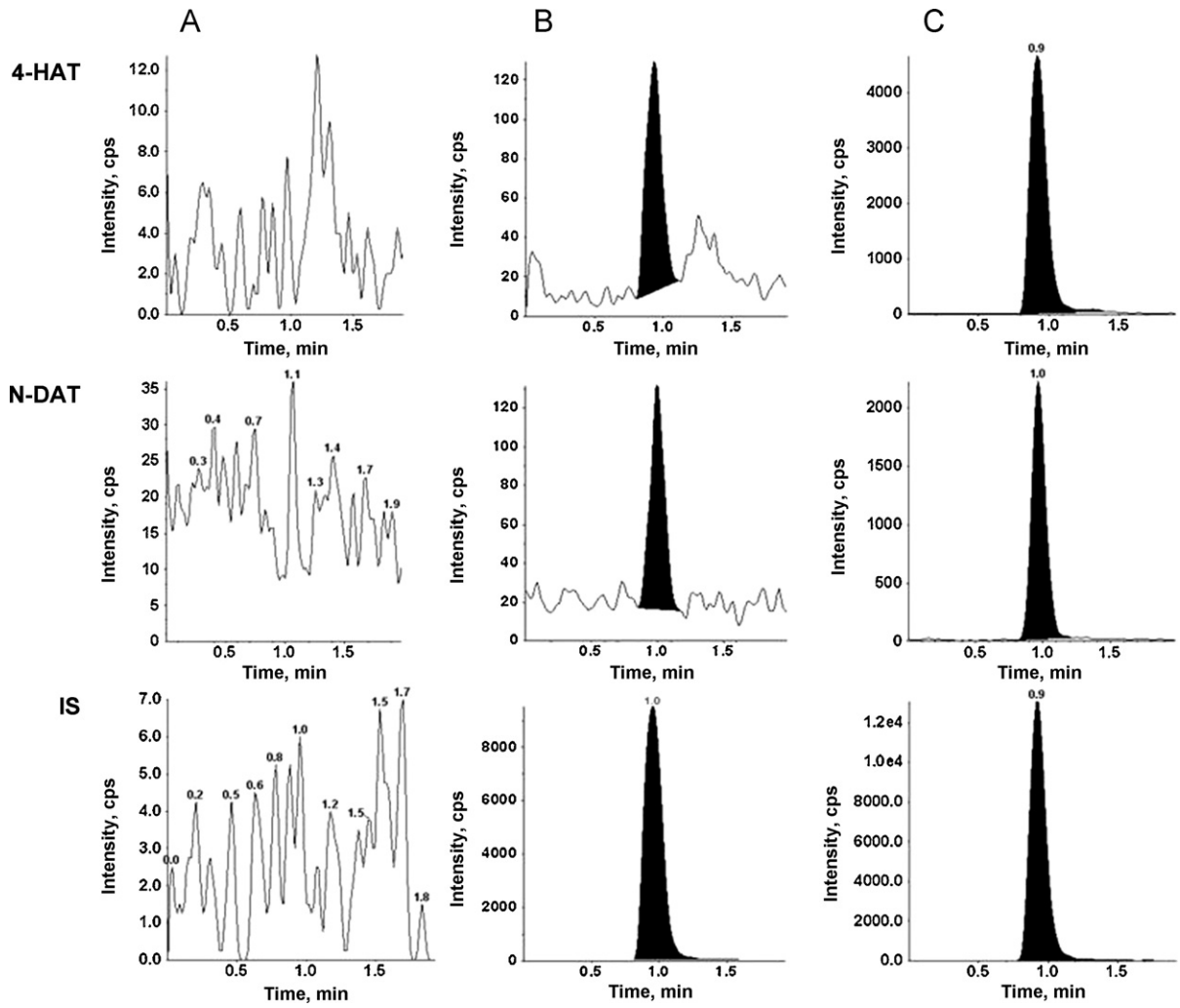

Fig. 2. Chromatograms of 4-hydroxyatomoxetine, *N*-desmethylatomoxetine and IS (metoprolol) in human plasma. (A) Blank human plasma; (B) blank human plasma spiked with 4-hydroxyatomoxetine (0.05 ng/mL), *N*-desmethylatomoxetine (0.1 ng/mL) and IS (300 ng/mL); (C) human plasma sample 6 h after administration of a single oral dose of 40 mg atomoxetine.

confirmed to be within ±20% limits for 16 (94%) and 14 (82%) of the reanalyzed samples, respectively (data not shown). These ISR results met the acceptance recommendation on reproducibility for incurred samples from the third AAPS/FDA Bioanalytical Workshop (Crystal City III) [16].

3.4. Pharmacokinetic application

Twelve Korean male volunteers with a mean age of 22.2 ± 2.0 years and a body mass index of 22.0 ± 1.7 kg/m^2 were enrolled in a pharmacokinetic study of atomoxetine. Our method was applied

Table 2
Stability of 4-hydroxyatomoxetine and *N*-desmethylatomoxetine in human plasma.

Targeted concentration (ng/mL)	4-Hydroxyatomoxetine			*N*-Desmethylatomoxetine		
	Measured concentration (ng/mL)	Accuracy (%)	Precision (CV%)	Measured concentration (ng/mL)	Accuracy (%)	Precision (CV%)
Post-preparative stability						
0.15 (0.3)	0.145 ± 0.010	96.4	7.1	0.289 ± 0.011	96.4	3.8
3	2.84 ± 0.17	94.6	6.1	2.98 ± 0.16	99.4	5.4
15	13.9 ± 0.3	92.9	1.8	14.6 ± 0.4	97.1	2.4
Short-term stability						
0.15 (0.3)	0.147 ± 0.004	98.2	2.6	0.293 ± 0.018	97.8	6.1
3	3.04 ± 1.07	101.2	5.6	2.84 ± 0.16	94.8	5.6
15	14.7 ± 0.4	97.8	2.8	14.6 ± 0.4	97.3	2.5
Freeze–thaw stability						
0.15 (0.3)	0.148 ± 0.007	98.7	4.7	0.303 ± 0.015	100.9	5.0
3	2.70 ± 0.16	90.1	5.9	2.87 ± 0.06	95.8	2.2
15	14.1 ± 0.4	94.0	2.6	14.5 ± 0.8	96.4	5.3
Long-term stability						
0.15 (0.3)	0.146 ± 0.010	97.3	6.5	0.306 ± 0.014	101.9	4.5
3	2.75 ± 0.14	91.7	5.0	3.00 ± 0.10	100.0	3.2
15	13.9 ± 0.4	92.9	2.9	14.6 ± 0.4	97.3	2.5

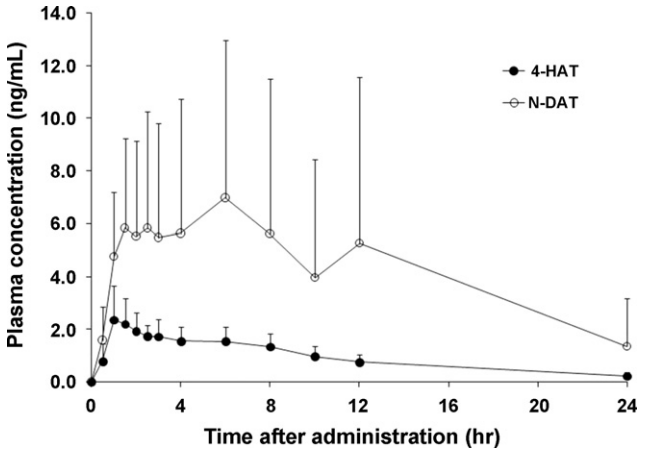

Fig. 3. Plasma concentration–time profile of 4-hydroxyatomoxetine (●) and *N*-desmethylatomoxetine (○) after administration of a single oral dose of 40 mg of atomoxetine in healthy male subjects ($n = 12$).

Table 3
Pharmacokinetic parameters of 4-hydroxyatomoxetine and *N*-desmethylatomoxetine after a single oral dose of 40 mg of atomoxetine in twelve healthy male subjects.

Pharmacokinetic parameters	4-HAT	N-DAT
C_{max} (ng/mL)	2.6 ± 1.1	7.8 ± 6.2
T_{max} (h)	1.9 ± 1.5	4.6 ± 3.6
$t_{1/2}$ (h)	6.4 ± 1.6	8.0 ± 7.4
AUC_t (ng h/mL)	21.7 ± 6.2	95.9 ± 97.5
AUC_{inf} (ng h/mL)	24.0 ± 7.6	117.7 ± 117.7

Each value represents the mean ± SD.

successfully to a pharmacokinetic study of 4-HAT and N-DAT after the administration of a single oral dose of 40 mg atomoxetine. Fig. 3 shows the mean plasma concentration–time profile of each analyte and Table 3 shows the pharmacokinetic parameters for the volunteers. There was considerable inter-individual variation in AUC_{inf} and C_{max} after oral administration of atomoxetine (Fig. 3 and Table 3). The AUC_{inf} values for 4-HAT and N-DAT were 14.9–45.1 ng h/mL and 25.2–358.5 ng h/mL, respectively, and the C_{max} values for 4-HAT and N-DAT were 1.4–5.0 ng/mL and 2.7–19.9 ng/mL, respectively. As mentioned above, atomoxetine is mainly metabolized by aromatic ring-hydroxylation, which is primarily mediated by CYP2D6 [4]. In addition, the CYP2C19 enzyme also affects the *N*-demethylation of atomoxetine [5]. These drug-metabolizing enzymes are known to be highly polymorphic and in previous studies, *CYP2D6* poor metabolizers had markedly increased plasma concentrations of atomoxetine and N-DAT and decreased plasma concentrations of 4-HAT and its glucuronide form, compared with *CYP2D6* extensive metabolizers [6,11]. Therefore, genetic polymorphisms in these drug-metabolizing enzymes are possible factors affecting the variability of the pharmacokinetics of 4-HAT and N-DAT, as well as atomoxetine.

4. Conclusions

We have developed and validated a simple, rapid and sensitive analytical LC–MS/MS method for the determination of two metabolites of atomoxetine in plasma samples with LLOQ values of 0.05 ng/mL for 4-HAT and 0.1 ng/mL for N-DAT. This method was sufficiently sensitive for analyzing the metabolites of atomoxetine in human plasma for up to 24 h after the administration of a single oral dose of 40 mg of atomoxetine. Although simultaneous determination of the parent drug, atomoxetine, was not achieved, the lower LLOQ, smaller plasma sample volume, and shorter run time (2.0 min) compared with other determinant methods [10,11] make our new method particularly suitable for use in routine assays.

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