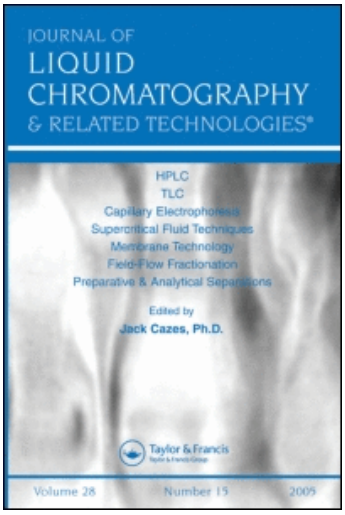

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A SIMPLE AND SPECIFIC HPLC METHOD FOR THE DETERMINATION OF ATOMOXETINE IN PHARMACEUTICALS AND HUMAN PLASMA

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A SIMPLE AND SPECIFIC HPLC METHOD FOR THE DETERMINATION OF ATOMOXETINE IN PHARMACEUTICALS AND HUMAN PLASMA

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□ *A liquid chromatographic method has been described for the determination of atomoxetine in pharmaceuticals and human plasma. Plasma samples were analyzed after a simple, one step protein precipitation with methanol, and chromatographic separation of atomoxetine and carbamazepine (internal standard) was carried out using the optimum mobile phase of a methanol/acetonitrile/phosphate buffer (10 mM, pH 3.0) (35:15:50, v/v/v). Limit of quantification values were 45.2 and 49.5 ng/mL for atomoxetine in the mobile phase and human plasma, respectively. Fully validated method is reproducible and selective for the determination of atomoxetine in pharmaceuticals and human plasma.*

Keywords atomoxetine, high performance liquid chromatography, human plasma, method validation, pharmaceutical analysis, protein precipitation

INTRODUCTION

Atomoxetine hydrochloride, (ATO, (-)-N-methyl-γ-(2-methylphenoxy) benzenepropanamine) (Figure 1), is a selective inhibitor of the presynaptic reuptake of norepinephrine. It has been approved by the FDA as the first non-stimulant drug for the treatment of attention deficit/hyperactivity disorder (ADHD). ADHD is the most common neurobehavioral disorder of childhood, and the symptoms of some patients persist to adulthood. It has been marked by symptoms of inattention, hyperactivity, and impulsiveness that impair academic and social functioning.[1,2]

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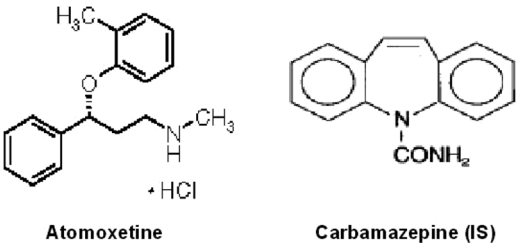

FIGURE 1 Chemical structures of atomoxetine and carbamazepine (IS).

ATO is predominantly metabolized by cytochrome P450 (CYP) 2D6 enzyme. It has been reported that maximum plasma concentrations of ATO have been observed in the range of 160–184 ng/mL in CYP2D6 extensive metabolizers and up to 925 ng/mL in genetically deficient CYP2D6 poor metabolizers after oral administration of 20 mg twice daily in adults.[1] Thus, for reliable therapeutic ATO monitoring, suitable and sensitive analytical methods are required.

Several chromatographic methods have been reported for the determination of ATO in human plasma including, high performance liquid chromatography (HPLC)[1–3] and liquid chromatography-tandem mass spectrometry (LC/MS/MS).[4] Also, chiral separation of ATO enantiomers is described.[5] A few HPLC methods have been reported for the determination of ATO in pharmaceutical formulations.[6–8] A HPTLC method is also available for the determination of ATO in pharmaceuticals.[9] These methods developed for the analysis of ATO in its dosage forms and also the chiral method, did not offer the adequate sensitivity for measuring ATO in plasma.[5–9] The methods reported for the determination of ATO in plasma offered adequate sensitivity; however, they involved liquid-liquid[1–3] or solid phase[4] extraction of ATO from the plasma samples prior to analysis. These time-consuming and expensive extraction procedures increase the overall analysis time and the cost of the analysis. The proposed method involves a simple plasma deproteination technique using one step protein precipitation with methanol. In addition, the procedure has minimal sample transfer steps, and it also does not harm the analyte.

In this study a rapid, sensitive, accurate, and precise HPLC method has been developed with a simple sample preparation procedure, short analysis time (less than 7 min), and an alternative C_8 separation column and fully validated. The proposed method has been successfully applied to the determination of ATO in pharmaceutical capsules and spiked human plasma.

EXPERIMENTAL

Chemicals

The standard ATO was supplied from Sigma-Aldrich (Steinheim, Germany). Its capsule preparation is Strattera®, a product from Lilly (Istanbul, Turkey) contained 25 mg active material, was purchased from a local drug store. All chemicals and reagents were the products of Merck Co. (Darmstadt, Germany) and were all of analytical-reagent grade, and, therefore, used with no further purification. Ultrapure water was used for the preparation of the solutions and mobile phase.

Chromatographic Conditions

The HPLC system consisted of a LC-10A pump equipped with a Rheodyne (Cotati, CA, USA) manual injector and a SPD-M10A diode array detector (Shimadzu, Kyoto, Japan). Data was processed with Class-LC10 software controlled by a CBM-10A communication module, all models of Shimadzu (Kyoto, Japan). Standard solutions and samples were injected into the 5 μL loop of the injector by means of a syringe with a 22-gauge needle. An Agilent (Eclipse, USA) XDB-C_8 column (3.5 μm spherical particle, 4.6 mm I.D. × 150 mm) was used throughout the study. B-220 model of sonicator from Branson (Danbury, CT, USA) was employed, and ultrapure water (18.2 μS/cm) was obtained from Millipore (Molsheim, France).

Preparation of Standard Solutions

A stock solution of ATO was prepared at a concentration of 1 mg/mL in methanol and serially diluted with water to give working standard solutions in the range of 49.5–1490 ng/mL. An IS solution of carbamazepine (1 mg/mL) was also prepared in methanol and used always at a fixed concentration of 495 ng/mL. Stock solutions and standards were stored in glass vials and they were covered with aluminum folia and kept at 4°C.

Chromatographic Separations

A number of mobile phase compositions were tested to find out the optimum conditions by changing organic solvent ratio, buffer pH, and concentration in the study. The optimized mobile phase consisted of methanol/acetonitrile/phosphate buffer (10 mM, pH 3.0) (35:15:50, v/v/v). It was degassed and filtered from 0.45-μm membrane filter under a negative

pressure before passing through the instrument. The flow-rate was 1.0 mL/min and the detector was set to 215 nm to detect the signals. The injection volume was 5 μL.

Method Validation

Validation of the proposed method was performed with respect to linearity, limit of detection (LOD), and limit of quantification (LOQ), accuracy and precision, stability, specificity, robustness, and ruggedness in accordance with ICH guidelines.[10] Calibration curves were obtained with six concentrations of the standard solutions of ATO in the range of 49.5–1490 ng/mL (n = 3). Linearity was evaluated by linear regression analysis using the least square regression method.

The precision of the method was evaluated as repeatability (intra-day) and intermediate precision (inter-day). Three different concentrations of ATO (99, 247.5, and 990 ng/mL) were analyzed in six independent series on the same day (intra-day precision) and on three consecutive days (inter-day precision).

The accuracy of the method was determined by analysis both of quality control samples prepared using standard ATO solution and of a solution of synthetic inactive ingredients (matrix) spiked with different known concentrations of ATO (within the calibration range). The matrix solution was prepared in methanol using common tablet (or capsule) excipients such as hydroxypropyl methyl cellulose (7%), lactose monohydrate (60%), magnesium stearate (1%), polyethylene glycol 4000 (5%), povidone (5%), maize starch (5%), talc (1%), and titanium dioxide (1%). Working standards of ATO were prepared at three concentrations (99, 247.5, and 990 ng/mL) in methanol and in the matrix solution. Percentage recoveries, percentage error, and percentage RSD values were used to express accuracy.

The specificity of the method was determined by analysis of ATO solutions (4.95 μg/mL) after these had been subjected to stress by treatment with 0.1 M HCl, 0.1 M NaOH, and 3% H_2O_2 at both room temperature and 60°C for different times (15, 30, 45, 60, 90, and 120 min). The samples were injected for HPLC analysis after dilution to 990 ng/mL.

The repeatability for injection was assessed by injecting standard solution of ATO (495 ng/mL) for six times. Data from replicate injections at this assay concentration were processed for calculations and 1% RSD was considered as limit.[11] The parameters tested for system suitability were capacity factor, resolution, tailing factor, theoretical plates, retention time, and percentage RSD of injection repeatability for the proposed method.

The robustness of the method was studied in triplicate for a concentration of 495 ng/mL by introducing small changes in the method conditions. The effect of these changes on the results was examined. Quantification was performed according to ATO-to-IS peak area ratio and system suitability data for ATO were calculated for each variation.

To assess the ruggedness of the method, a solution of ATO at a concentration of 495 ng/mL was analyzed by two different analysts. Data were treated to calculate RSD % and the results were compared by t and F test.

The stability of standard solutions of ATO was evaluated by triplicate analysis of solutions at two concentrations, 495 and 990 ng/mL, under different conditions. To assess short-term stability, standard solutions of ATO were stored at room temperature for 24 hr. Long-term stability was assessed after storage of the solutions in a freezer at −20°C for 20 days. To assess freeze and thaw stability, ATO solutions were stored at −20°C for 24 hr and thawed to room temperature, the cycle being repeated three times, and then analyzed.

Sample Preparation

Ten Strattera® capsules (each containing 25 mg ATO) were weighed and finely powdered in a mortar. The net weight of each capsule was calculated. A sufficient amount of drug equivalent to the average weight of capsule content was accurately weighed and methanol was added to dissolve the active material (n = 6). It was sonicated for 10 min and then the solution was centrifuged at 5000 rpm for 10 min. The supernatant was diluted to obtain the concentrations that would be in the available range of calibration studies.

Plasma Sample Preparation

Blood samples were withdrawn from healthy volunteers and plasma was separated by centrifuging for 10 min at 5000 rpm. Certain amount of ATO (containing 49.5–792 ng/mL) was transferred into 100 µL plasma at six concentrations for three times and the contents of the tube were vigorously shaken. Methanol was used for deproteinization of plasma proteins at a ratio of 1 part plasma to 4 parts methanol and it was vigorously mixed using a shaker. The precipitated proteins were separated by centrifuging for 10 min at 5000 rpm. Clear supernatant was diluted with water containing 495 ng/mL IS and directly injected to HPLC system in the same experimental conditions.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

ATO is a basic drug with a pKa of 9.23.[12] HPLC analysis of basic samples can be troublesome because of poor peak shape and reduced column efficiency originating from silanol interactions.[13] In one of the previous HPLC studies of ATO, an amine modifier was used, whereas, in another study, both ion-pairing reagent and amine modifier were used to solve problems originating from silanol interactions.[3,8] Although, using amine modifiers like triethylamine, seems to be a good approach to mask unbonded silanols, it slows column equilibration down when changing mobile phases, and also both chemicals increase the cost of the analysis. As a result, their use must be the last approach after all of the others have been tried.[13]

The solvent type and strength (B %) in the mobile phase have a great effect on selectivity and sensitivity of the drugs to be analyzed. Initially 50% methanol and then, 40% acetonitrile was investigated to obtain good peak shape and suitable retention time of ATO. As it was reported that the organic solvent mixture was a good approach to improve separation characteristics of basic analytes,[13,14] the acetonitrile-methanol mixtures of different compositions (20:30%, 10:35%, 20:20%, 15:25%, 30:15%, 15:35%) were investigated at the similar solvent strength. Methanol was preferred for use at higher ratios according to acetonitrile due to the greater solubility of phosphate buffer in methanol-water mixtures.[13] It was also reported that methanol, as a lipophilic alcohol, was well adsorbed on silica, which performed a homogenous stationary phase and improved selectivity and peak characteristics.[14] The best compromise between column efficiency, peak morphology, and run time was obtained with the methanol-acetonitrile ratio of 35–15%.

Utilization of phosphate buffer is the most common strategy for the analysis of ATO to improve peak characteristics and also optimize the analysis time.[2,6,7] Initially, the mobile phase without phosphate buffer was used and the retention time of ATO was increased to 25 min with poor peak morphology originating from the lack of pH optimization. Then, the mobile phase including phosphate buffer at different pH values (in the pH range of 2.5–5.5) was investigated for the chromatographic separation of ATO. This pH range was selected mainly in accordance with the knowledge of ATO and basic sample characteristics. It has been reported that pH should be varied, out of the range of pKa ± 2, to obtain more rugged separation and for retention to be unaffected by small changes in pH. Also, mobile phases with low pH ($2.0 < \text{pH} < 3.5$) are proposed for basic samples against poor peak shape and reduced column efficiency originating from silanol

interactions.[13] The retention time and peak area of ATO were increased with the increasing pH of the mobile phase, but at high pH values (pH = 5.5), the plate number was extremely decreased. Considering the reasonable retention time and the theoretical plate number of ATO with minimum tailing factor (t = 1.37), pH 3.0 was chosen as the optimum pH of the phosphate buffer.

The other important parameter of the optimization of mobile phase is the concentration of the buffer. The concentration of the phosphate buffer was investigated in the range of 0–40 mM. The retention time of ATO and peak morphology was not changed excessively with the increasing buffer concentration. In considering the column safety and to minimize the cost of analysis, 10 mM was chosen as the optimum concentration of phosphate buffer.

As a consequence, the optimum conditions were determined as a mobile phase containing methanol/acetonitrile/phosphate buffer (10 mM, pH 3.0) (35:15:50, v/v/v), flow rate of 1.0 mL/min, 5 μL loop volume, detection wavelength of 215 nm. The system suitability data for ATO in these conditions are presented in Table 1.

Internal standard (IS) is usually used in HPLC methods in order to increase the accuracy and precision. Carbamazepine was selected as the appropriate IS because of its reasonable retention time and the resolution with ATO peak in spite of its quite different structure (Figure 1). Thus, high method precision and accuracy were obtained using IS.

Method Validation

Precision

Statistical evaluation of results from determination of precision, which showed intra-day and inter-day RSD, were in the ranges 0.48–1.86% and 1.27–1.72%, respectively. The RSD % values obtained were less than 2% and, therefore, acceptable in analytical points' of view, exhibiting the sufficient method precision.[10,11]

TABLE 1 System Suitability Data for ATO

Parameters	Observed Values	Recommended Values
Retention time	6.05	
Capacity factor (k')	2.73	>2
Tailing factor (T)	1.37	≤2
Resolution (Rs)	2.66	>2
Theoretical plates (N)	7071.00	>2000
RSD (%) (for Ret.Time)	0.18	≤1

TABLE 2 Calibration Results of ATO with their Statistical Analysis (day = 3; n = 6)

	Mobile Phase	Human Plasma
Linearity Range (ng/mL)	49.5–1490	49.5–792
Slope	0.00064	0.00051
Intercept	0.018	0.064
Correlation Coefficient	0.9993	0.9992
SE of slope	0.0000068	0.0000065
SE of intercept	0.004	0.003
LOD (ng/mL)	14.92	16.34
LOQ (ng/mL)	45.20	49.50

Linearity

Calibration curves for standard ATO in mobile phase were constructed by plotting concentration versus the ratio of peak areas of ATO and IS, and showed good linearity in the range of 49.5–1490 ng/mL. The results of linear regression analysis of the curves were presented in Table 2. High correlation coefficient was obtained and the intercept of the curve was not significantly different from zero. Resolution was always good in the studied linearity range.

Certain analytical parameters such as limit of detection (LOD) and limit of quantification (LOQ) were estimated [standard deviation of regression equation/slope of regression equation] by multiplying with 3.3 and 10, respectively, and are presented in Table 2.

Accuracy

Accuracy was tested as described in experimental section and was evaluated as percentage error [(found concentration-spiked concentration)/spiked concentration] × 100%, and precision was evaluated by determination of the coefficient of variation (CV %, RSD %, [SD/mean × 100]) at low, central and high concentrations in the linearity range. The acceptance criteria are no higher than 2% deviation from the nominal value for accuracy and no more than 2% RSD for precision.[10,11] Recovery was almost 100% for the drug substance and the drug product and accuracy was much better than the acceptance criterion. The same concentrations were used to evaluate precision as repeatability. The RSD % values were also much better than the acceptance criterion, showing the precision of the method was sufficient as seen in Table 3.

Specificity

The specificity of the method was tested as described in the experimental section and it was seen that ATO did not undergo degradation in the acid solution at room temperature or when heated to 60°C for

TABLE 3 The Results of Method Accuracy of Standard ATO and ATO Spiked Matrix

	Added ATO (ng/mL)	Found ATO (ng/mL) (mean ± SD, n = 6)	Recovery (%)	Accuracy (%)	RSD (%)
Quality control solutions	99.0	103.07 ± 1.10	102.04	2.04	1.07
	247.5	249.06 ± 1.76	100.63	0.63	0.71
	990.0	995.18 ± 6.86	100.52	0.52	0.69
Matrix solutions spiked with ATO	99.0	100.97 ± 1.58	101.99	1.99	1.57
	247.5	247.68 ± 1.89	100.07	0.07	0.76
	990.0	1000.08 ± 8.07	101.02	1.02	0.81

120 min. Although, the base stressed samples showed no degradation at ambient temperature and at 60°C after 120 min, there was a large peak at solvent front that was getting smaller from time to time. The H_2O_2 stressed samples showed approximately 10% degradation at ambient temperature, whereas it showed approximately 23–25% degradation at 60°C after 120 min resulting in a large peak at 1.5 min as seen in Figure 2c. None of these peaks seen in the H_2O_2 or base stressed sample chromatograms

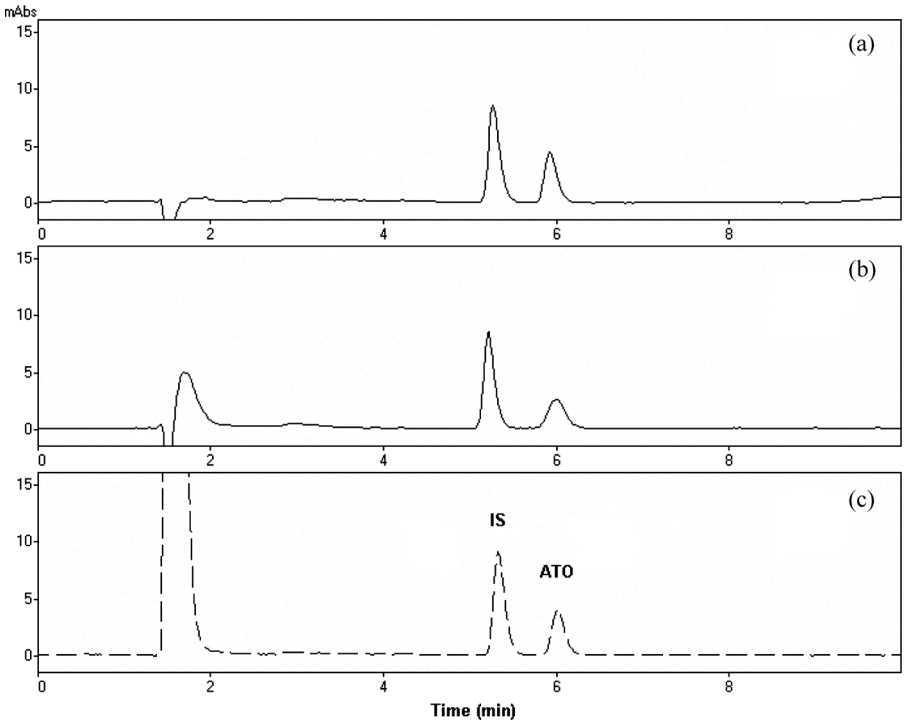

FIGURE 2 Specificity of the method at 60°C in 120 min: (a) ATO in 0.1 M HCl; (b) ATO in 0.1 M NaOH; and (c) ATO in 3% H_2O_2.

showed any interference with ATO peak as confirmed by UV spectrum with a photodiode array detector.

Testing was also performed with the inactive ingredients in the capsules to ensure none of these interfered with the peaks of interest. The data indicated that these ingredients did not interfere with ATO and IS peaks, so the specificity of this method was considered sufficient as seen in Figure 3.

Robustness

The recovery (%) compared to optimum conditions and the system suitability parameters such as retention time, plate number, tailing factor, and resolution were calculated after introducing small deliberate changes in the developed HPLC method. Low RSD values (<2) were obtained indicating the robustness of the method as seen in Table 4.[15]

Ruggedness

Low RSD values of 0.73 and 0.86% (<2) for peak-area ratios were obtained when the same solution of ATO was analyzed by two analysts

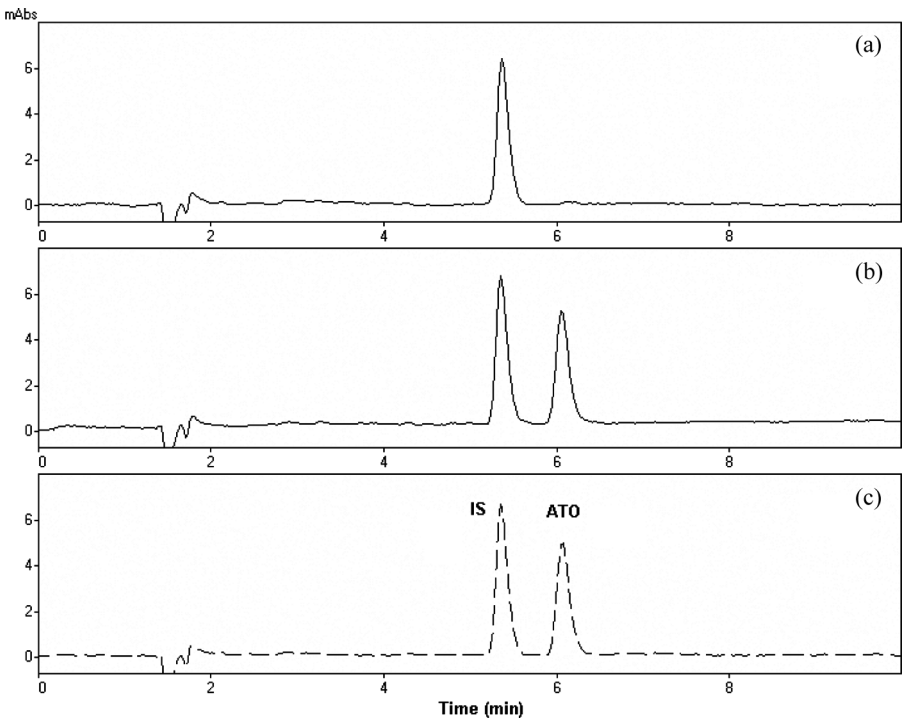

FIGURE 3 Chromatograms obtained from ATO and IS: (a) capsule matrix without ATO; (b) capsule matrix spiked with ATO (990 ng/mL); and (c) capsule assay.

TABLE 4 Robustness of the HPLC Method (mean ± SD, n = 3, 495 ng/mL ATO)

	Recovery (%)	Retention Time (min)	Plate Number	Tailing Factor	Resolution (*R*s)
Phosphate buffer pH					
pH 2.7	101.81 ± 1.80	6.28 ± 0.02	7337.7 ± 30.1	1.35 ± 0.01	2.91 ± 0.02
pH 3.3	97.47 ± 0.62	5.83 ± 0.04	7038.7 ± 41.59	1.39 ± 0.02	2.53 ± 0.01
Buffer concentration					
9 mM	99.78 ± 0.59	5.87 ± 0.02	7052.7 ± 39.3	1.35 ± 0.02	2.40 ± 0.03
11 mM	101.65 ± 1.03	6.12 ± 0.06	7169 ± 84.7	1.38 ± 0.01	2.70 ± 0.04
Amount of organic solvent (%)					
45%	97.47 ± 1.17	9.02 ± 0.13	10031 ± 136.2	1.32 ± 0.02	5.11 ± 0.02
55%	103.10 ± 1.81	4.45 ± 0.02	6298.3 ± 50.54	1.52 ± 0.01	1.27 ± 0.02
Wavelength					
212 nm	106.09 ± 1.97	6.04 ± 0.01	7031.3 ± 86.74	1.37 ± 0.01	2.67 ± 0.02
218 nm	103.39 ± 0.59	6.05 ± 0.01	7003 ± 6.00	1.35 ± 0.02	2.68 ± 0.01
Flow rate					
0.9 mL/min	98.33 ± 0.71	6.67 ± 0.01	7624.7 ± 94.52	1.41 ± 0.03	2.74 ± 0.01
1.1 mL/min	95.58 ± 0.70	5.43 ± 0.004	6653.7 ± 71.07	1.43 ± 0.02	2.48 ± 0.02

(n = 5). High reproducibility and insignificant differences between the two analysts' results were obtained at the 95% probability level for *t* and *F* tests of significance $0.12 < 2.78$ and $1.42 < 6.39$, respectively, indicating the method was rugged.[15]

Stability

During assessment of stability, recovery was in the range 96.08–100.54% and RSD was in the range 0.93–1.72% as seen in Table 5. ATO standard solutions were therefore stable under all the conditions tested.

Application of the Method to Pharmaceuticals

The application of the developed method for the determination of ATO was performed in capsules containing 25-mg active material as

TABLE 5 Stability of ATO Under Different Storage Conditions (mean ± SD, n = 3)

		Short-Term Stability (24 h, Room Temperature)		Long-Term Stability (20 days, −20°C)		Freeze-Thaw Stability (3 cycles)	
Theoretical Concentration (ng/mL)		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Standard solution	495	100.27 ± 1.25	1.24	97.84 ± 1.17	1.19	96.08 ± 1.66	1.72
	990	98.97 ± 0.92	0.93	100.54 ± 1.39	1.38	99.86 ± 1.03	1.03
Human Plasma	198	95.19 ± 1.58	1.65	101.25 ± 1.08	1.07	96.70 ± 1.14	1.18
	594	99.32 ± 1.43	1.44	101.90 ± 1.64	1.61	92.66 ± 1.60	1.73

described in the experimental section. The peaks of capsule samples carried the characteristics of standard ATO and no interference originated from the matrix was observed. The capsule content was $101.64 \pm 0.97\%$ (mean $\pm$ SD, $n = 6$) with the RSD of 0.95%; these values are within the limits recommended in USP.[16]

Application of the Method to Human Plasma Samples

The plasma proteins are precipitated by the addition of methanol (1:4, v/v) in order to obtain maximum efficiency.[17] The mixture is centrifuged at 5000 rpm and the supernatant is directly injected for analysis. The chromatograms of blank plasma and ATO (792 ng/mL) spiked plasma samples are presented in Figure 4. The peak shape and characteristics in spiked plasma samples were not different from the standards. The ratios of peak areas of ATO and IS in plasma were statistically evaluated for three concentrations (99, 198, and 792 ng/mL) on three days and the results showed RSD % of 1.15–2.77 indicating good intra-day precision and RSD % of

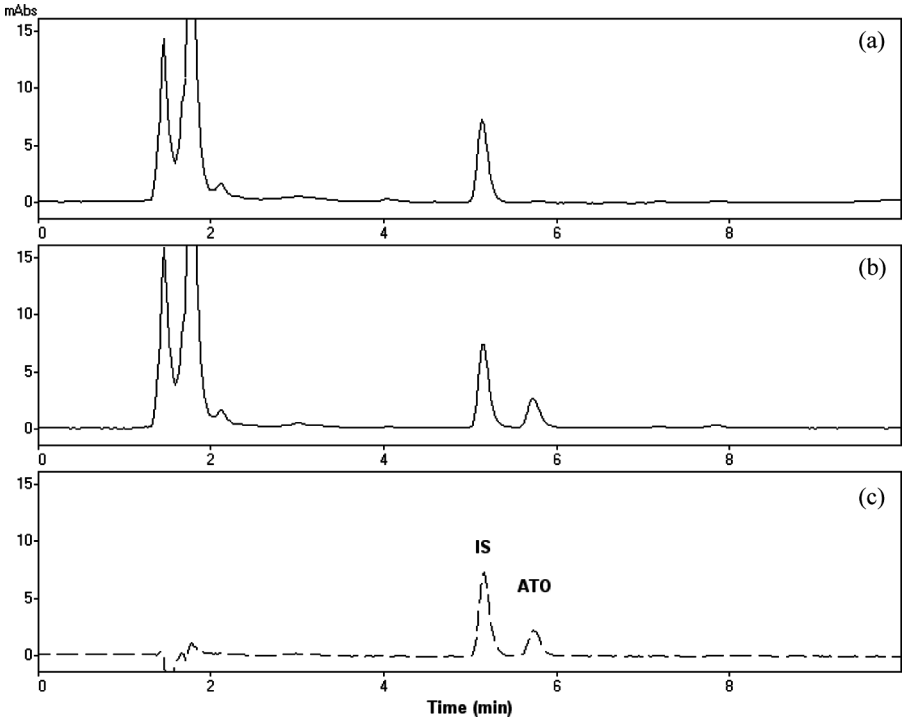

FIGURE 4 Chromatograms obtained from analysis of ATO (792 ng/mL) in plasma under the optimum conditions: (a) blank plasma; (b) plasma sample spiked with ATO; and (c) standard ATO and IS in mobile phase.

TABLE 6 Method Recovery and Precision for ATO in Human Plasma

Nominal Concentration (ng/mL)		Measured Concentration (ng/mL)	Recovery (%)	RSD (%)
Intra-day (n = 6)	99	86.48 ± 2.40	85.62	2.77
	198	165.62 ± 4.09	83.65	2.47
	792	633.13 ± 7.30	79.94	1.15
Inter-day (n = 18)	99	82.82 ± 1.42	81.99	1.72
	198	168.73 ± 2.11	85.22	1.25
	792	661.47 ± 10.53	83.52	1.59

1.25–1.72 indicating good inter-day precision as seen in Table 6.[18] The linearity of the method was established in pooled human plasma in the concentration range of 49.5–792 ng/mL for ATO at six concentrations for three days. The mean result of three sets of linearity with high correlation coefficients was given in Table 2. The low standard error values of the slope and the intercept in plasma samples also showed the precision of the proposed method.

Specificity was also performed with the same column using blank plasma to assure that the matrix could be interfered with the relevant peaks. The data indicated that the matrix did not interfere with the ATO and IS peaks, so the specificity was considered sufficient for the application of the method to plasma analysis as seen in Figure 4.

The stability of ATO in human plasma was evaluated at two concentrations of 198 and 594 ng/mL under the same conditions with standard solutions. The results presented in Table 5 indicated that ATO was found to be stable in human plasma under all mentioned conditions.

The absolute recovery of ATO in spiked plasma samples was about 83%. Application of the proposed method to plasma samples resulted in detection and quantification limits of 16.34 and 49.5 ng/mL, respectively, thus enabling therapeutic drug monitoring by use of a simple, selective, and rapid sample preparation technique.

CONCLUSIONS

A reversed phase HPLC method has been established for analysis of ATO in pharmaceutical preparations and human plasma. The retention time of ATO (6.05 min) enables rapid analysis of the drug, which is important for routine analysis. The method was completely validated and the results obtained confirmed its suitability for purpose.

This HPLC method is more sensitive than other methods used for pharmaceutical analysis[6–9] and the sensitivity is comparable with that of previous HPLC method for which the sensitivity was 50 ng/mL.[3] However,

Guo et al. have reported a more sensitive HPLC method with LOD of 2.5 ng/mL.[2] Although the methods of LC/MS/MS and HPLC-fluorescence are more sensitive, the cost of analysis and troublesome derivatization steps limit the use of these methods.[1,4] Moreover, all the previously reported methods for the quantitation of ATO in biological fluids require time-consuming and expensive extraction steps.[1–4] Proposed single step deproteinization procedure with methanol is quite simple, inexpensive, and also very rapid to eliminate proteins from the sample.

Also, the developed HPLC method provides higher recovery values (80–85%) compared to the values of other HPLC methods which are almost about 70–75%.[1–3] However, Mullen et al. obtained better recovery values which are about 97% for plasma samples.[4]

HPLC methods have several advantages, for example, low detection and quantification limits, small sample volumes, and high accuracy and reproducibility. Specificity tests were successfully performed and ATO could be effectively separated from its degradation products. As a conclusion, the developed HPLC method for the determination of ATO in both pharmaceuticals and human plasma is simple, specific, reliable, and cost effective and can be proposed for use in routine analysis laboratories and quality control purposes.

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REFERENCES

1. Zhu, H. J.; Wang, J. S.; Donovan, J. L.; DeVane, C. L.; Gibson, B. B.; Markowitz, J. S. Sensitive Quantification of Atomoxetine in Human Plasma by HPLC with Fluorescence Detection Using 4-(4,5-diphenyl-1H-imidazole-2-yl) Benzoyl Chloride Derivatization. *J. Chromatogr. B* **2007**, *846*, 351–354.
2. Guo, W.; Li, W.; Guo, G.; Zhang, J.; Zhou, B.; Zhai, Y.; Wang, C. Determination of Atomoxetine in Human Plasma by a High Performance Liquid Chromatographic Method with Ultraviolet Detection Using Liquid-Liquid Extraction. *J. Chromatogr. B* **2007**, *854*, 128–134.
3. Patel, C.; Patel, M.; Rani, S.; Nivsarkar, M.; Padh, H. A New High Performance Liquid Chromatographic Method for Quantification of Atomoxetine in Human Plasma and Its Application for Pharmacokinetic Study. *J. Chromatogr. B* **2007**, *850*, 356–360.
4. Mullen, J. H.; Shugert, R. L.; Ponsler, G. D.; Li, Q.; Sundaram, B.; Coales, H. L.; Yakupkovic, J. E.; LeLacheur, R. M.; Wheeler, W. J.; Belas, F. J.; Sauer, J. M. Simultaneous Quantification of Atomoxetine as Well as İts Primary Oxidative and O-glucuronide Metabolites in Human Plasma and Urine Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS). *J. Pharm. Biomed. Anal.* **2005**, *38*, 720–733.

5. Sellers, J. A.; Olsen, B. A.; Owens, P. K.; Gavin, P. F. Determination of the Enantiomer and Positional Isomer Impurities in Atomoxetine Hydrochloride with Liquid Chromatography Using Polysaccharide Chiral Stationary Phases. *J. Pharm. Biomed. Anal.* **2006**, *41*, 1088–1094.
6. Kamat, S. S.; Choudhari, V. B.; Vele, V. T.; Prabhune, S. S. RP-LC Determination of Atomoxetine HCl from İts Pharmaceutical Dosage Form. *Chromatographia* **2008**, *67*, 143–146.
7. Nagaraju, V.; Crouch, M. A. Development and Validation of a Rapid HPLC Assay for the Simultaneous Determination of Three Psychoanaleptic Drugs in Pharmaceutical Formulations. *J. Liq. Chromatogr. Related Technol.* **2008**, *31*, 722–732.
8. Jagadeesh, B. V. V. S.; Raju, S. S.; Rao, V. J.; Rao, J. V. L. N. S. Reverse Phase HPLC Analysis of Atomoxetine in Pharmaceutical Dosage Forms. *Asian J. Chem.* **2009**, *21*, 829–833.
9. Kamat, S. S.; Vele, V. T.; Choudhari, V. C.; Prabhune, S. S. HPTLC Determination of Atomoxetine Hydrochloride from İts Bulk Drug and Pharmaceutical Preparations. *Asian J. Chem.* **2008**, *20*, 5409–5413.
10. International Conference on Harmonization (ICH) Q2(R1) *Validation of Analytical Procedures: Text and Methodology, Harmonized Tripartite Guideline*, ICH: 2005.
11. Shabir, G. A. Validation of High-Performance Liquid Chromatography Methods for Pharmaceutical Analysis: Understanding the Differences and Similarities Between Validation Requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *J. Chromatogr. A* **2003**, *987*, 57–66.
12. Material Safety Data Sheet. http://www.ehs.lilly.com/msds/strattera.pdf. Accessed October 27 2010.
13. Snyder, L. R.; Kirkland, J. J.; Glajch, J. L. *Practical HPLC Method Development*, Wiley: New York, USA, 1997; 292–349.
14. Başcı, N. E.; Temizer, A.; Bozkurt, A.; Isimer, A. Optimization of Mobile Phase in the Separation of *β*-blockers by HPLC. *J. Pharm. Biomed. Anal.* **1998**, *18*, 745–750.
15. Dejaegher, B.; Vander Heyden, Y. Ruggedness and Robustness Testing. *J. Chromatogr. A* **2007**, *1158*, 138–157.
16. The United States Pharmacopeia (USP-XXX), Marck Printing Co.: Easton, PA, USA, 2007.
17. Wells, D. A. *High Throughput Bioanalytical Sample Preparation Methods and Automation Strategies*, Elsevier: UK, 2003; 199–207.
18. Shah, V. P.; Midha, K. K.; Findlay, W. A.; Hill, H. M.; Hulse, D. J.; McGilveray, I. J.; Mc Key, G.; Miller, K. J.; Patnaik, R. N.; Powell, M. L.; Tonelli, A.; Viswanathan, C. T.; Yacobi, A. Bioanalytical Method Validation-a Revisit with a Decade of Progress. *Pharm. Res.* **2000**, *17*, 1551–1557.