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Spectrophotometric and spectrofluorimetric determination of atomoxetine in pharmaceutical preparations

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Visible spectrophotometric and spectrofluorimetric methods were developed for the determination of atomoxetine in pharmaceutical preparations. The spectrophotometric method was based on a nucleophilic substitution reaction of atomoxetine with 1,2-naphthoquinone-4-sulphonate (NQS) in an alkaline medium to form an orange-colored product. The absorbance-concentration plot is rectilinear over the range 5–40 μg mL^{-1}. The limits of detection and quantification were calculated to be 0.02 μg mL^{-1} and 0.06 μg mL^{-1}, respectively. The spectrofluorimetric method was based on the derivatization reaction of 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) with atomoxetine to produce a fluorescent derivative. The formed highly fluorescent derivative that was measured at 462 nm after excitation at 533 nm. The fluorescence-concentration plot is rectilinear over the range 10–500 ng mL^{-1}. The limits of detection and quantification were calculated to be 0.19 ng mL^{-1} and 0.57 ng mL^{-1}. The analytical performance of both methods was fully validated, and the results were satisfactory. The methods have been successfully applied for the determination of the studied drug in capsules and the results obtained ware in good agreement with those obtained by the reference method.

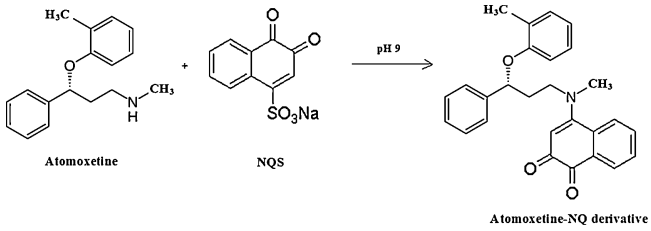
1. Introduction

Atomoxetine hydrochloride (ATO) (-)-N-methyl-γ-(2-methylphenoxy)benzenepropanamine hydrochloride is a selective noradrenaline reuptake inhibitor, recently approved for the treatment of attention-deficit/hyperactivity disorder (Garnock-Jones et al. 2009).

A few methods have been published for the analysis of ATO in biological fluids and in pharmaceutical preparations. Some of these are high performance liquid chromatographic (HPLC) methods (Zhu et al. 2007; Patel et al.2007; Guo et al. 2007; Dogrukol-Ak et al. 2010), liquid chromatography–mass spectrometry (Choong et al. 2009), liquid chromatography tandem mass spectrometry(LC/MS/MS) (Mullen et al. 2005), gas chromatography-electron capture (Farid et al. 1985), gas chromatography-mass spectroscopy (Garside et al. 2006).

A few methods have been developed for the determination of ATO in pharmaceutical preparations; these include HPLC (Kamat et al. 2008; Nagaraju et al. 2008; Patel et al. 2010) voltammetry (Pérez-Ortiz et al. 2010) and UV spectrophotometry (Koradia et al. 2009).

The visible spectrophotometry and spectrofluorimetry of ATO has not been described in the literature. In this study, ATO has been derivatized with NQS and NBD-Cl for the first time. Additionally, an official method for the determination of ATO in pharmaceutical preparation has not been described in any pharmacopoeia. The proposed method is selective, simple, and sensitive has been successfully applied to the determination of ATO in pharmaceutical preparations.



Scheme 1: Reaction of ATO with NQS

2. Investigations, results and discussion

2.1. Spectrophotometric method

NQS has been used as a chromogenic and fluorogenic reagent for primary and secondary amines (Pesez and Bartos 1974). The proposed method was based on a nucleophilic substitution reaction of ATO with NQS. The NQS reacted with ATO at the free secondary group as represented in the Scheme 1.

The NQS with ATO product is orange colored exhibiting a maximum absorption at 449 nm (Fig. 1).

To optimize the assay variables, the effects of concentration of NQS, pH, reaction time, temperature and the extracting solvent on the absorbance of the derivative formed were studied. The effect of reagent volume was studied over a range of 0.1–1 mL where maximum color intensity was obtained upon using 0.5 mL of NQS reagent. The effect of pH on the absorbance of the derivative formed was also studied. The absorbance of the ATO-NQS derivative solution was investigated over the pH range 8–10. The optimum absorbance was achieved at pH 9. In order

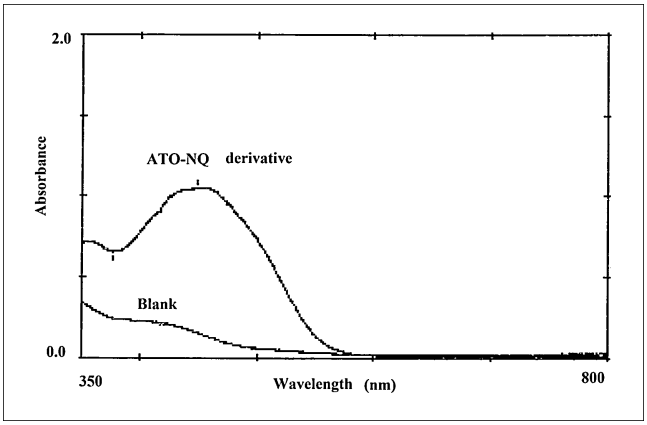


Fig. 1: Absorption spectra on the reaction product of ATO with NQS

to examine the effect of temperature and reaction time on the absorbance of the derivative, the above mentioned procedure was carried out at different temperatures (40, 50, 60, 70 and 80 °C) using a thermostatic water bath. Maximum and constant absorbance was obtained at 70 °C after 40 min (Fig. 2). Different solvents were tested: methanol, water, ethanol, chloroform and dichloromethane where chloroform was found to be the most suitable solvent that provides maximum absorbance.
The color formed under the above mentioned optimum conditions was stable for at least 2 h.
Job's method of continuous variations was employed; a 3.4×10^{-3} M solution of ATO and 3.4×10^{-3} M solution of NQS under consideration were used. The reagent-drug ratio was found to be 1:1 (NQS:ATO).

2.2. *Spectrofluorimetric method*

NBD-Cl, an activated halide derivative was first introduced as a fluorogenic reagent for the determination of secondary and primary amines in low amounts (Imai et al. 1989). ATO contains a secondary aliphatic amino group that was found to react with NBD-Cl in an alkaline buffered medium yielding a highly yellow fluorescent product that exhibited its highest fluorescence intensity at 462 nm after excitation at 533 nm (Fig. 3). Scheme 2 shows the derivatization reaction of the ATO with NBD-Cl.
Besides, different experimental parameters affecting the intensity of ATO-NBD derivative were investigated to find out the optimum parameters.
The influence of the concentration of NBD-Cl was studied using different volumes (25–200 μL) of 0.25% solution of the reagent. The maximum fluoresccence intensity was obtained with 100 μL of the reagent. The maximum intensity of the associated compound was observed at pH 9. It is known that the maximum association between NBD-Cl and the aimed compound is realized under basic conditions. However, NBD-Cl is also hydrolyzed in alkaline solution. Thus, the system stabilized by acidifying the reaction mixture to pH 2 (by adding 100 μL 0.1N HCl) before measurement (Imai et al. 1989).
The influence of temperature and its duration on the intensity of the ATO-NBD derivative were also examined. Five different temperatures in the range of 40–80 °C were investigated for NBD derivation. The best results were obtained at 70 °C after 20 min. The effect of time and temperature versus intensity of ATO-NBD compound is demonstrated in Fig. 4.
Different solvents were tested: methanol, ethanol, ethyl acetate, chloroform and dichloroform where methanol was found to be the most suitable solvent that provides maximum fluorescence intensity.
The color formed under the abovementioned optimum conditions was stable for at least 2 h.
For the study, NBD-Cl and ATO solutions, prepared as 3.4×10^{-4} M, were mixed in varying volume ratios in which the total volume of the mixtures was kept at 10 mL. Under the optimum conditions, the stoichiometry of the reaction between ATO and NBD-Cl was investigated by Job's. The reagent-drug ratio was found to be 1: 1(NBD-Cl:ATO).

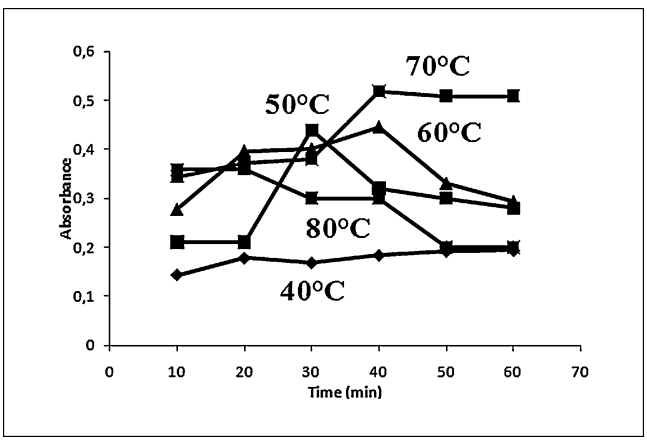


Fig. 2: Effect of time and temperature on the reaction of ATO with NQS

2.3. *Method validation*

The proposed method was validated according to the International Conference on Harmonization (ICH) Guidance Documents testing (ICH topic Q2B 1996). The system linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, recovery, robustness and specificity parameters were analyzed for the validation testing.
Calibration curves of ATO were linear over the concentration range of 5–40 μg mL^{-1} for the spectrophotometric method and 10–500 ng mL^{-1} for the spectrofluorimetric method. These values are much lower than those obtained by many other methods (Patel et al. 2010; Kamat et al. 2008; Koradia et al. 2009).
The limit of detection (LOD) and limit of quantification (LOQ) of the drug for the proposed methods were determined using calibration standards. LOD and LOQ were calculated from the equation of [(standard deviation of intercept)/(slope of regression)by multiplying 3.3 and 10, respectively.
LOD and LOQ were 0.02 μg mL^{-1} and 0.06 μg mL^{-1} for the spectrophotometric method. LOD and LOQ were 0.19 and 0.57 ng mL^{-1} for the spectrofluorimetric method. Thus, the method is much more sensitive than most of the reported methods (Patel et al. 2010; Kamat et al. 2008; Koradia et al. 2009).
Accuracy and precision were checked by three times analysis for three different concentrations of pure samples. The relative standard deviation (RSD %) and relative mean error (RME %) values of intra-day and inter-day studies showed good precision and accuracy. The results are summarized in Table 1.
Recovery studies were carried out by the standard addition method. From the amount of drug found, the percentage recovery was calculated by the following equation:

$$Recovery(\%) = Ct - Cu/Ca \times 100$$

where *C*t is the total concentration of the analyte found, *C*u the concentration of the analyte present the formulation, and Ca is the concentration of the pure analyte added to the formulation.
The recovery values were 98.04–99.07% for the spectrophotometric method and 98.12–99.16% for the spectrofluorimetric method (Table 2).

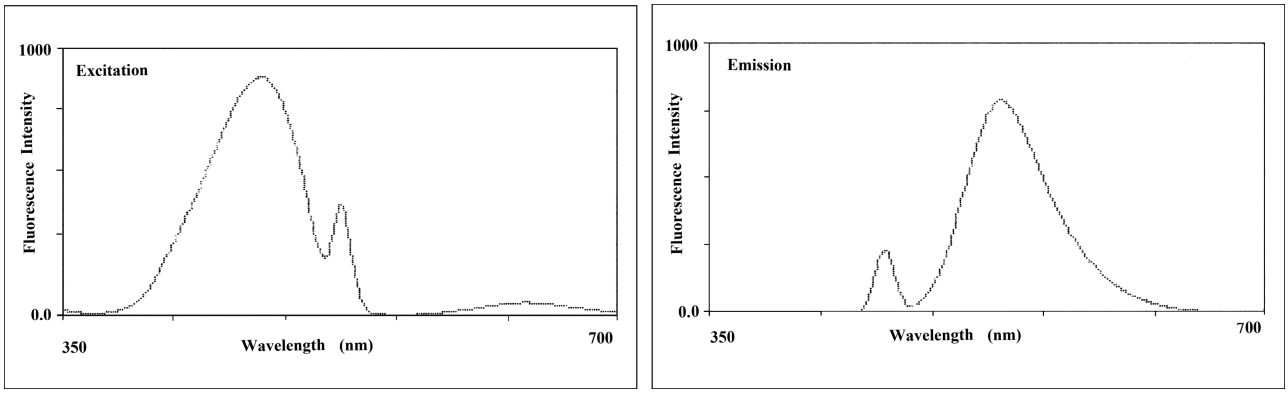
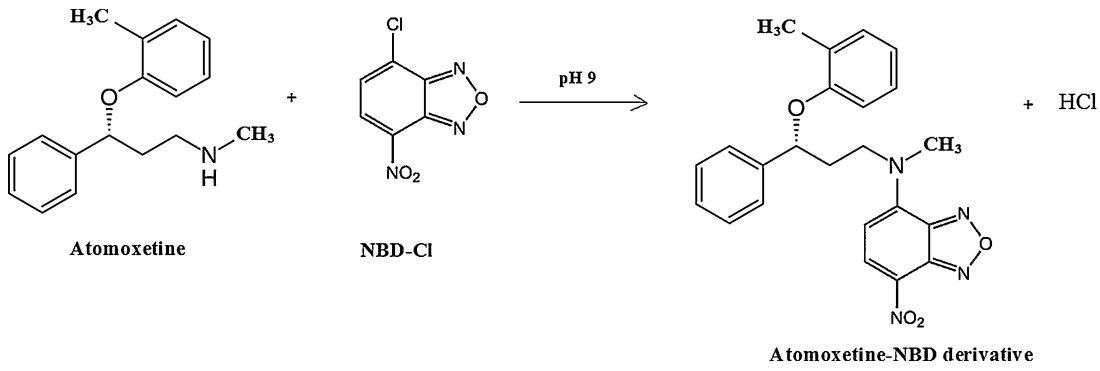


Fig. 3: Excitation and emission spectrums on the reaction product of ATO with NBD-Cl



Scheme 2: Reaction between ATO and NBD-Cl

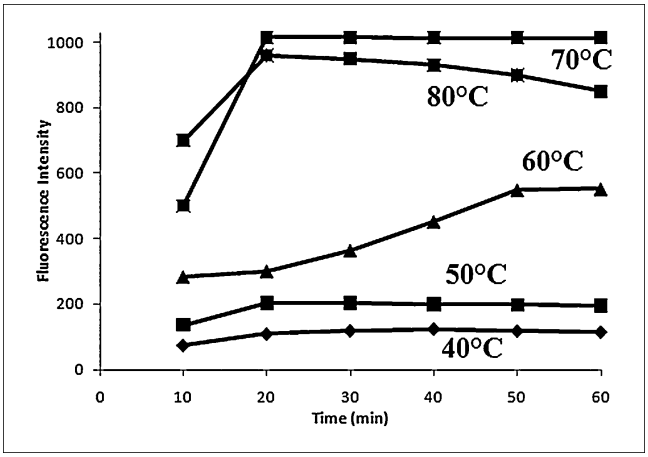


Fig. 4: Effect of time and temperature on the reaction of ATO with NBD-Cl

The variables evaluated in the study include reagents concentration (±0.1), pH (±0.1), temperature (±1 °C) and time (±2 min). It was found that small variations in the method variables did not significantly affect the procedure.

The specificity of the methods was investigated by observing any interference encountered from common excipients of the pharmaceutical preparations such as magnesium stearate, hydroxypropyl methyl cellulose, polyethylene glycol 4000, lactose monohydrate, talc and titanium dioxide. It was found that these compounds did not interfere with the results of the spectrophotometric and spectrofluorimetric methods.

2.4. Application to pharmaceutical capsules

The spectrophotometric and spectrofluorimetric methods have been successfully applied to the determination of the ATO in pharmaceutical capsules. The results obtained are shown in (Table 3). This result was compared with that obtained from the reference method (Koradia et al. 2009) by statistical analysis with respect to the accuracy (by *t*-test) and precision (by *F*-test). No significant differences were found.

Table 1: Intra- and inter-day precision and accuracy of the assay ATO

Spectrophotometric method				Spectrofluorimetric method			
Added concentration ($\mu g mL^{-1}$)	Found concentration ($\mu g\,mL^{-1}$) mean ± SD	Precision (RSD%)	Accuracy (RME%)	Added concentration ($ng\,mL^{-1}$)	Found concentration ($ng\,mL^{-1}$) mean±SD	Precision (RSD%)	Accuracy (RME%)
Intra-day				*Intra-day*			
5.0	5.30 ± 0.12	2.26	+6.0	10	10.42 ± 0.10	0.95	+4.20
20	20.18 ± 0.13	0.64	+0.9	30	300.28±0.37	0.12	+0.09
40	40.13 ± 0.14	0.35	+0.33	500	500.14±0.11	0.02	+0.03
Inter-day				*Inter-day*			
5.0	5.42±0.25	4.16	+8.40	10	10.45±0.16	1.53	+4.50
20	20.21±0.12	0.59	+1.05	300	300.32±0.39	0.12	+0.11
40	40.16±0.15	0.37	+0.40	500	500.15±0.12	0.2	+.0.03

Table 2: Recovery results of ATO from capsule matrix

Concentration of drug in formulations(μg/mL) or (mg/mL)	Concentration of pure drug added (± SD)	Recovery (%)
Spectrophotometric method		
5.0	5.0 ± 0.11	98.04
5.0	15.0 ± 0.14	98.79
5.0	35.0 ± 0.16	99.07
Spectrofluorimetric method		
5.0	25 ± 0.10	98.12
5.0	195 ± 0.41	99.10
5.0	495 ± 0.11	99.16

3. Experimental

3.1. Materials and measurements

ATO was purchased from Sigma–Aldrich (Steinheim, Germany). Strattera® 10 mg capsules were obtained from a local pharmacy. The NQS and NBD-Cl were purchased from Merck (Darmstadt, Germany). All of the chemicals and solvents were of analytical grade.

3.2. Apparatus

UV-160A (Shimadzu, Kyoto, Japan) ultraviolet-visible spectrophotometer with matched 1 cm glass cells was used for all spectrophotometric measurements. Fluorescence spectrum was recorded by a RF-1501 Model a Shimadzu (Kyoto, Japan) spectrofluorimeter.

3.3. Solutions

A stock solution of ATO was prepared in methanol at a concentration of 1 mg mL^{-1}. Standard solution of ATO was prepared in methanol at a concentration of 100 μg mL^{-1}. NQS solution of 0.5% (w/v) was prepared in water. NBD-Cl was freshly prepared by dissolving 25 mg in 10 mL methanol.
A borate buffer (0.1 M) was prepared by dissolving 0.620 g of boric acid and 0.750 g of potassium chloride in 100 mL water. The pH was adjusted to 9.0 with 0.1 M sodium hydroxide solution and the volume was made up to 200 mL with water.

3.4. Procedures

3.4.1. Spectrophotometric method

Aliquots of ATO standard solution covering the concentration range of 5–40 μg mL^{-1} were transferred into a series of 12 mL stoppered tubes; 0.5 mL of borate buffer (pH = 9.0) was added, followed by 0.5 mL of NQS (0.5%) solution and mixed well. The reaction was allowed to proceed at 70 °C in a water bath for 40 min; then cooled and 100 μL of 0.1 M HCl was added. The content of tube was extracted three times with 2 mL of chloroform. The organic layer was transferred into another clean 10 mL volumetric flasks and diluted to the mark with chloroform.

3.4.2. Spectrofluorimetric method

Aliquots of ATO standard solution covering the concentration range of 10–500 ng mL^{-1} were transferred into a series of 12 mL stoppered tubes; 100 μL of borate buffer (pH = 9.0) was added, followed by 100 μL of NBD-Cl (0.25%) solution and mixed well. The reaction was allowed to proceed at 70 °C in a water bath for 20 min; then cooled and 100 μL of 0.1 M HCl was added. The mixture was transferred into another clean 10 mL volumetric flasks and diluted to the mark with methanol.

Table 3: Assay results of tablets containing 10 mg ATO

Drug (Stratterra 10 mg)	%Recovery ± SD[a]	t^b	F^b
Spectrophotometric method	98.87 ± 0.19	0.12	2.32
Spectrofluorimetric method	99.20 ± 0.21	0.09	1.91
Comparison Method (Koradia et al. 2009)	98.76 ± 0.29		

[a] Values are mean six determinations
[b] The tabullated of *t* and *F* at %95 confidence limit are 2.23 and 5.05, respectively.

3.4.3. Procedure for the pharmaceutical preparations

Ten capsules were accurately weighed and finely powdered rapidly. An amount of the powder equivalent to 100 mg of ATO was transferred into a 100 mL volumetric flask containing about 50 mL of methanol. The mixture was then introduced into an ultrasonic bath for 30 min and then completed to volume with methanol and filtered, discarding the first 10 mL.
Finally, a 10 mL aliquot of this solution was diluted to 100 mL with methanol in a volumetric flask. This solution was analyzed according to the general analytical procedure. The content of the capsule was calculated using the corresponding regression equation of the calibration graph.

References

Choong E, Rudaz S, Kottelat A, Guillarme D, Veuthey JL, Eap CB (2009) Therapeutic drug monitoring of seven psychotropic drugs and four metabolites in human plasma by HPLC–MS. J Pharm Biomed Anal 50: 1000–1008.

Dogrukol-Ak D, Yeniceli D (2010) A simple and specific HPLC method for the determination of atomexetine in pharmaceuticals and human plasma. J Liq Chromatogr Related Technol 33: 1745–1759.

Garnock-Jones KP. Keating GM (2009) Atomoxetine: a review of its use in attention-deficit/hyperactivity disorder in children and adolescents. Paediatr Drugs 11: 203–206.

Garside D, Ropero-Miller J, Riemer EC (2006) Postmortem Tissue Distribution of Atomoxetine Following Fatal and Nonfatal Doses-Three Case Reports. J Forensic Sci 51: 179–182.

GuoW, Li W, Guo G, Zhang J, Zhou B, Zhai Y, Wang C (2007) Determination of atomoxetine in human plasma by a high performance liquid chromatographic method with ultraviolet detection using liquid–liquid extraction. J Chromatogr B 854: 128–134.

Farid NA, Bergstrom RF, Ziege EA, Parli CJ, Lemberger L (1985) Single-dose and steady-state pharmacokinetics of tomoxetine in normal subjects. J Clin Pharmacol 25: 296–301.

ICH topic Q2B. Validation of analytical procedures: methodology. *ICH Harmonized Tripartite Guidelines*. ICH, Geneva, Switzerland, November 6, 1996, CPMP/ICH/281/95.

Imai K, Uzu S, Toyoóka T (1989) Fluorogenic reagents, having benzofurazan structure, in liquid chromatography. J Pharm Biomed Anal 7: 1395–1403.

Kamat SS, Choudhari VB, Vele VT, Prabhune SS (2008) RP-LC Determination of Atomoxetine HCl from its Pharmaceutical Dosage Form. Chromatographia 67: 143–146.

Koradia SK, Shah PT, Rana RR, Vaghani SS, Pandey S, Jivani NP (2009) Spectrophotometric determination of atomoxetine hydrochloride from its pharmaceutical dosage forms. Asian J Research Chem 2: 258–259.

Mullen JH, Shugert RL, Ponsler GD, Li Q, Sundaram B, Coales HL, Yakupkovic JE, Lelacheur RM, Wheeler WJ, Belas FJ, Sauer JM (2005) Simultaneous quantification of atomoxetine as well as its primary oxidative and *O*-glucuronide metabolites in human plasma and urine using liquid chromatography tandem mass spectrometry (LC/MS/MS). J Pharm Biomed Anal 15: 720–33.

Nagaraju V, Crouch AM (2008) Development and validation of a rapid HPLC assay for the simultaneous determination of three psychoanaleptic drugs in pharmaceutical formulations. J Liq Chromatogr Relat Technol 31: 722–732.

Patel C, Patel M, Rani S, Nivsarkar M, Padh H (2007) A new high performance liquid chromatographic method for quantification of atomoxetine in human plasma and its application for pharmacokinetic study. J Chromatogr B 850: 356–360.

Patel SK, Patel N J (2010). Development and validation of a stability-indicating RP-HPLC method for determination of atomoxetine hydrochloride in tablets. J AOAC Int 93: 1207–1214.

Pérez-Ortiz M, Muñoz C, Zapata-Urzúa C, Álvarez-Lueje A (2010) Electrochemical behavior of atomoxetine and its voltammetric determination in capsules.Talanta 82: 398–403.

Pesez M, Bartos J (1974) Colorimetric and Fluorimetric Analysis of Organic Compounds and Drugs, Marcel Dekker Inc., New York.

Zhu HJ, Wang JS, Donovan JL, Devane CL, Gibson BB, Markowitz JS (2007) 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 846: 351–354.