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SHORT COMMUNICATION

Spectrophotometric Quantification of Atomoxetine Hydrochloride Based on Nucleophilic Substitution Reaction with 1,2-Naphthoquinone-4-Sulfonic Acid Sodium Salt (NQS)

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ABSTRACT

Objectives: A simple, sensitive, selective, and cost-effective colorimetric method has been established for the quantitative estimation of atomoxetine hydrochloride in bulk and formulation.

Materials and Methods: It was established based on the visible reaction between atomoxetine hydrochloride and 1,2-naphthoquinone-4-sulfonic acid sodium salt in a basic medium (potassium hydroxide). The resulting orange colored chromogen exhibited an absorption maximum at 474 nm.

Results: Based on the optimization studies, distilled water as the solvent, 1% *w/v* potassium hydroxide (2 mL), and 0.3% *w/v* 1,2-naphthoquinone-4-sulfonic acid sodium salt (2 mL) were used in the method. The developed method was validated *per* the International Council for Harmonization (ICH) guidelines. The linearity was found at a concentration of 10-50 µg/mL. The method showed a good correlation between the concentration of atomoxetine hydrochloride and its absorbance. The correlation coefficient (r^2) of 0.999 evidenced the same. The limits of detection and quantification were 0.20 and 0.606 µg/mL, respectively, for atomoxetine hydrochloride. The accuracy and precision of the method were also evaluated and the results obtained were within the acceptance criteria (relative standard deviation % < 2.00). The percentage assay of atomoxetine hydrochloride proved to be 101.52, which is in accordance with its label claim.

Conclusion: The developed method is non-complex and can be effectively employed in the analytical practices of atomoxetine hydrochloride in pharmaceutical dosage forms.

Key words: Colorimetry, atomoxetine hydrochloride, accuracy, precision, linearity

INTRODUCTION

Atomoxetine hydrochloride is chemically known as (*R*)-*N*-methyl-3-phenyl-3-(*o*-tolyloxy) propylamine hydrochloride (Figure 1).[1] As a norepinephrine reuptake inhibitor, it is used in the management of attention/deficit hyperactivity disorder. Atomoxetine hydrochloride is the subject of a monograph published in the Indian Pharmacopoeia. Its titration with acetous perchloric acid in the presence of acetous mercuric acetate and further potentiometric determination at the end-point were described in Indian Pharmacopiea.[2]

Several methods have been presented in the literature for the determination of atomoxetine hydrochloride. A few of them include visible spectrometric methods involving oxidative

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coupling with sodium per-iodate and folic reagent,[3] nucleophilic substitution with 1,2-naphthoquinone-4-sulfonic acid sodium salt (NQS) in alkaline medium[4] and condensation with vanillin/*para*-dimethylamino benzaldehyde,[5] ultraviolet spectrophotometric methods using solvents, such as double distilled water,[6] acetonitrile,[7] 0.05 N hydrochloric acid,[8] spectrofluorimetric methods based on the emergence of binary complex with eosin Y in Teorell-Stenhagen buffer[9] and reaction with 4-chloro-7-nitro-2,1,3-benzoxadiazole in chloroform.[4] Furthermore, reverse-phase high-performance liquid chromatographic methods with combinations of stationary and mobile phases,[10-15] ultra-performance liquid chromatographic method,[16] and liquid chromatography-tandem mass spectroscopic method[17] have also been mentioned in the literature.

A literature survey revealed that even though sophisticated instrument techniques are available for atomoxetine hydrochloride (reverse phase high-performance liquid chromatography, ultra-performance liquid chromatography, and liquid chromatography-tandem mass spectroscopy), their use is limited as they require costly instruments, solvents/reagents, skilled operators, and tedious extraction protocols. It was identified that the colorimetric method reported used borate buffer-chloroform-NQS in alkaline medium and the color was developed with the aid of heat (70 °C) after 40 min of addition of the reagents.[4] NQS is one of the extensively used chromogenic reagents for the determination of primary or secondary amine-containing drugs.[18] Colorimetry is persistently competitive with chromatographic techniques due to its simplicity, sensitivity and selectivity, cost-effectiveness, fair accuracy, precision, and easy access in most quality control laboratories for pharmaceutical analysis.[19]

Keeping these facts in mind, the present work was attempted by dissolving the analyte in the most economical and easily available solvent, *i.e.*, distilled water, and using NQS as the reagent. Furthermore, the reaction was performed at room temperature. The details of the methods and materials adopted and the results obtained are discussed in the following chapters.

MATERIALS AND METHODS

Materials and instrumentation

Atomoxetine hydrochloride standard (Hetero Drugs Pvt. Ltd., India) and marketed tablets (Axepta-10, contains 10 mg of atomoxetine hydrochloride *per* tablet) from a local drugstore were used as received. We used analytical grade chemicals and reagents for establishing the analytical method. Colorimetric measurements were performed using a double-beam ultraviolet-visible spectrophotometer (Shimadzu 1800, Japan). The standard statistical functions were computed using the options available in MS-EXCEL.

Reagents and standard solutions

NQS reagent (0.3% w/v)

An accurately weighed NQS (0.30 g) was transferred into a 100 mL volumetric flask and dissolved in an appropriate volume of distilled water.

Potassium hydroxide solution (1% w/v)

In a 100 mL volumetric flask, accurately measured potassium hydroxide (1.00 g) was transferred, and the volume was made with distilled water.

Standard stock solution of atomoxetine hydrochloride

Atomoxetine hydrochloride (1,000 µg/mL) was produced by solubilizing a precisely weighed substance (10.00 mg) in distilled water contained in a volumetric flask (10 mL). Transferred 1 mL into another 10 mL volumetric flask from the above stock, and the volume was finally made with distilled water to acquire 100 µg/mL as the end concentration.

Analysis of atomoxetine hydrochloride

Aliquots of the standard drug solution of atomoxetine hydrochloride (100 µg/mL) ranging from 1 to 5 mL were transferred to a group of 10 mL volumetric flasks. They were shaken vigorously after adding potassium hydroxide solution (1% *w/v*, 2 mL) and NQS reagent (0.3% *w/v*, 2 mL). The volume was adjusted with water to prepare a batch of analytical solutions comprising 10-50 µg/mL of atomoxetine hydrochloride. The absorbance of the resulting colored complex was recorded after 20 min at λ_{max} 474 nm against the corresponding reagent blank. Beer-Lambert's plot was used to compute the amount of atomoxetine hydrochloride.

Analytical method optimization

The optimum concentration of potassium hydroxide and NQS reagent, reaction time, and mole ratio were studied during method development, and the details are provided in the results and discussions.

Analytical method validation

Validation of the method was attempted by checking linearity, accuracy, precision, sensitivity, and robustness by adopting the procedures stated in the International Council for Harmonization (ICH) guidelines.[20]

Linearity

Aliquots of stock solution (100 µg/mL) of atomoxetine hydrochloride were conveyed to a set of volumetric flasks to obtain final concentrations in the span of 10-50 µg/mL. Potassium hydroxide solution (1% *w/v*, 2 mL) and NQS reagent (0.3% *w/v*, 2 mL) were added to the above analyte solution, and the volume was made using distilled water. Using an appropriate blank, the absorbance of colored chromogen was recorded after 20 min at λ_{max} 474 nm. The calibration curve was developed by plotting the absorbances against the drug concentrations.

Accuracy

Standard solutions of atomoxetine hydrochloride were added at 80, 100, and 120% levels to pre-quantified sample solutions of atomoxetine hydrochloride (20 µg/mL). Each sample was tripled at the individual level. The atomoxetine hydrochloride quantity was estimated using acquired absorbance values in the regression equation.

Precision

The intra-day and inter-day precisions of the proposed colorimetric technique were established by estimating the responses in six replicates of atomoxetine hydrochloride (30 µg/mL) on the corresponding day and three distinct days in a week, respectively. The outcomes are described in terms of percentage relative standard deviation (% RSD).

Sensitivity and robustness

The lowest detectable amount [limit of detection (LOD)] and the lowest quantifiable amount [limit of quantitation (LOQ)] in the method were determined using samples containing very low concentrations of atomoxetine hydrochloride, as stated in the ICH guidelines. The LOD was calculated as 3.3 multiplied by the ratio of standard deviation and slope. Similarly, the LOQ was calculated by 10 multiplied by the ratio of the standard deviation and slope. Sandell's sensitivity of atomoxetine hydrochloride was computed from the quotient of molecular weight and molar absorptivity. Furthermore, the method was also tested with minor modifications in the concentration of NQS and potassium hydroxide to ensure robustness and the resulting responses were recorded.

Assay of atomoxetine hydrochloride in the marketed tablets

The marketed tablets (Axepta-10 containing 10.00 mg atomoxetine hydrochloride) were precisely measured and ground to a fine powder. The powder analogous to 10.00 mg analyte was dispersed in distilled water (10 mL) to produce 1,000 µg/mL and then sonicated for 5 min. The resulting mixture was then shaken vigorously and filtered using Whatman's filter paper (no: 41). Clear filtrate (1 mL) was shifted into a 10 mL volumetric flask and diluted with distilled water up to the mark. From this 100 µg/mL solution, a 3 mL portion was moved to another 10 mL volumetric flask, 2 mL of 1% *w/v* potassium hydroxide and 2 mL of 0.3% *w/v* NQS were added. Final fabrication was performed with distilled water. This solution was used to estimate atomoxetine hydrochloride after 20 min. The absorbance was recorded at 474 nm using an appropriate reagent blank. The amount of atomoxetine hydrochloride was computed by incorporating responses into the regression equation, with correction for dilution, and the results were statistically validated.

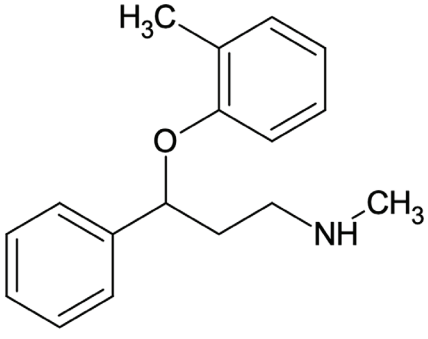

Figure 1. Structure of atomoxetine hydrochloride

RESULTS AND DISCUSSION

Development and optimization of the analytical method

This colorimetric method was developed on the basis of the reaction between NQS and atomoxetine under basic conditions at ambient temperature. The reaction yielded orange-colored chromogen, which displayed a maximum absorbance at 474 nm after 20 min of addition of the reagent (Figure 2). The probable reaction of NQS with atomoxetine hydrochloride in the basic medium is depicted in Figure 3.

Selection of the solvent

The ultraviolet (UV) absorbance of atomoxetine hydrochloride was determined by dissolving the analyte in different solvents such as distilled water, methanol, ethanol, and acetonitrile (Supplementary Table 1). Distilled water was selected as the solvent based on its high absorbance, low cost, and ease of availability.

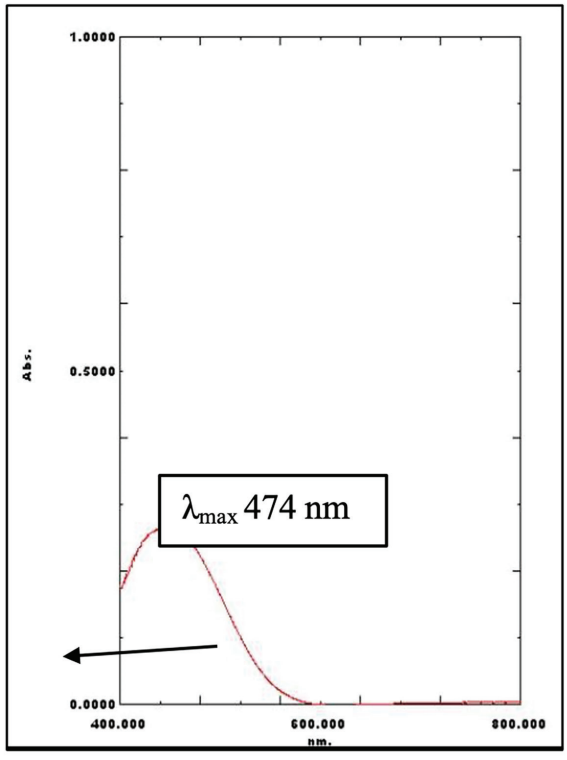

Figure 2. 2 UV absorption spectrum of atomoxetine hydrochloride at λ_{max} 474 nm in distilled water (10 µg/mL)

UV: Ultraviolet

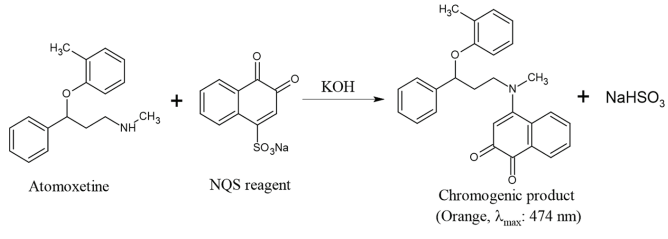

Figure 3. Probable reaction between atomoxetine hydrochloride and NQS

NQS: 1,2-Naphthoquinone-4-sulfonic acid sodium salt

Table 1. Accuracy data of the analytical method

% Level	Formulation (µg/mL)	Conc. of std. spiked (µg/mL)	Amount found (AM ± SD, n: 3)	Bias	% Recovery	% RSD*
80	20	16	35.283 ± 0.301	0.174	98.00	0.85
100	20	20	40.451 ± 0.313	0.181	101.13	0.77
120	20	24	43.524 ± 0.675	0.389	98.92	1.55

AM: Arithmetic mean, SD: Standard deviation, Bias: Standard deviation/square root of sample size, RSD: Relative standard deviation
*Acceptance criteria: % RSD should not exceed 2.00

Optimization of the concentration of potassium hydroxide and NQS

Atomoxetine hydrochloride solution (100 µg/mL, 1 mL) was shifted to a volumetric flask (10 mL), mixed with various concentrations of potassium hydroxide (2 mL, 0.5, 1.0, 1.5, and 2.0%, *w/v*), NQS reagent (0.3 and 0.5%, *w/v*) and finally diluted with distilled water. Each analyte solution was produced by changing only one variable (either concentration of potassium hydroxide or NQS) at a time, and the response of the resulting solutions was recorded at 474 nm after 20 min (Figures 4 and 5). The results indicated that 0.3% (*w/v*) concentration of NQS and 1% (*w/v*) concentration of potassium hydroxide showed constant and highest absorbance among all the combinations. Therefore, the same were selected for further investigation.

Selection of the wavelength

The analyte solution (10 µg/mL in distilled water) along with the above mentioned reagents was scanned in the visible range (400-800 nm), and the spectrum obtained is shown in Figure 2. From the spectrum, λ_{max} was found to be 474 nm for atomoxetine hydrochloride. The absorbance of the analyte solutions at the same wavelength was recorded throughout this investigation.

Optimization of the reaction time

Color development was monitored at discrete time intervals (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min) to optimize the reaction time. Maximum absorbance was noted after 20 min (Figure 6). Stable color development was noticed up to 6 h under optimized conditions.

Stoichiometry of the reaction

The continuous variation method was used to investigate the stoichiometry of the reaction. Samples were prepared with equimolar amounts of atomoxetine hydrochloride (3.43 x 10^{-5} M) and NQS, while other reaction conditions were the same as mentioned earlier. The drug and reagent (NQS) were assorted to produce different mole ratios (0.2:0.8, 0.4:0.6, 0.5:0.5, 0.6:0.4, and 0.8:0.2, respectively). The stoichiometric relationship between the two variables is shown in Figure 7 and a mole ratio of 0.5:0.5 gave the highest absorbance value.

Analytical method validation

The response of atomoxetine hydrochloride at 474 nm was deliberated in 10-50 µg/mL concentration range. The data for the calibration curve and regression analysis of the calibration curve are shown in Figure 8. The study resulted in a correlation coefficient (r^2) value of 0.999. From the results, it was observed that with an increase in the concentration of atomoxetine hydrochloride, the absorbance also increased linearly.

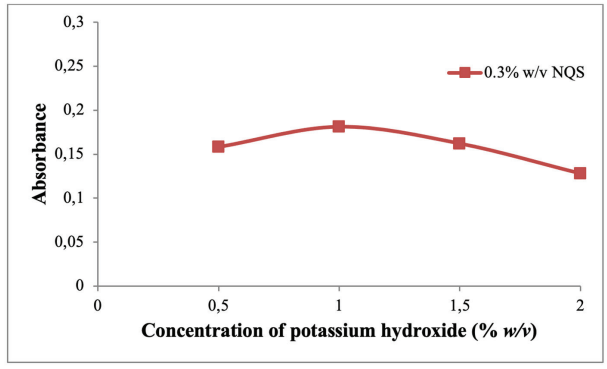

Figure 4. Effect of the concentration of potassium hydroxide and 0.3% (*w/v*) NQS in X-axis scale as- 0, 0.5, 1, 1.5 and 2, Y-axis scale as 0, 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3

NQS: 1,2-Naphthoquinone-4-sulfonic acid sodium salt

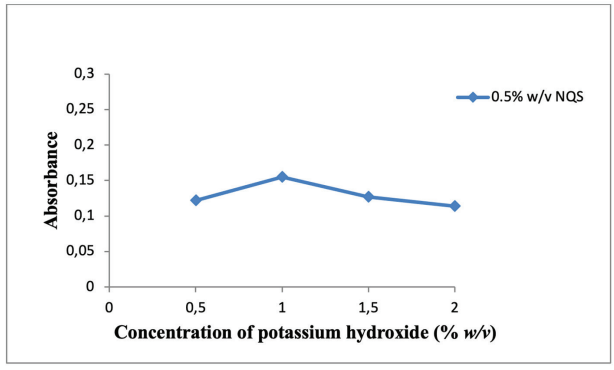

Figure 5. Effect of the concentration of potassium hydroxide and 0.3% (*w/v*) NQS, X-axis scale as - 0, 0.5, 1, 1.5, and 2, Y-axis scale as - 0, 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3

NQS: 1,2-Naphthoquinone-4-sulfonic acid sodium salt

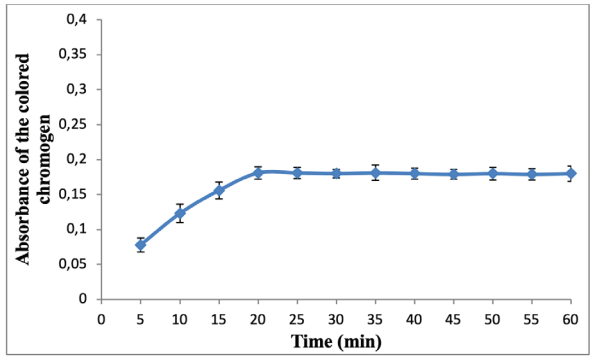

Figure 6. Effect of time on the stability of the colored complex at 474 nm

Y-axis scale as 0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, and 0.4

The exactness of the method was confirmed by spiking the standard analyte in 80, 100, and 120% concentrations to the fixed concentration of atomoxetine hydrochloride in the formulation (20 µg/mL). The details are presented in Table 1. They were found to be significant under specification limits, with percent recovery of 98.00-101.13% and the percentage RSD was found to be < 2.00 for the drug. The percentage recovery of atomoxetine hydrochloride for the three levels was found to be satisfactory.

Atomoxetine hydrochloride at a concentration of 30 µg/mL was used to determine the repeatability (intra-day precision) and intermediate precision of the method. The data obtained in the precision studies are provided in Table 2. The precision of the method was confirmed by observing a % RSD value less than 2.00 in both studies.

The sensitivity of the methodology was resolute in terms of LOD and LOQ, which were calculated as 0.2 and 0.606 µg/mL, respectively. The Sandell's sensitivity of the method was determined and calculated as 0.0553 µg/cm^2. The influence of minor dissimilarities in the concentrations of potassium hydroxide and NQS (1.0 ± 0.1 and 0.3 ± 0.1%, *w/v*, respectively) was studied to ensure the robustness of the method. The results revealed that these dissimilarities did not affect the absorbance of the formed colored complex to a large extent.

Assay of atomoxetine hydrochloride in the marketed tablets

The assay of commercial tablets was performed to determine the reliability of the proposed method. The results obtained were compared against the corresponding labeled claim. The amount of atomoxetine hydrochloride was determined as 10.152 ± 0.049 mg and the % assay was computed as 101.52%. The results denoted that the assay results agreed with the respective labeled claim and that there was no interference of excipients from the formulation at the determined wavelength (Table 3).

The details of the current and literature colorimetric and UV spectrophotometric methods developed for atomoxetine hydrochloride are provided in Table 4. The colorimetric method described by Ulu[4] used borate buffer and chloroform as solvents, and the color was developed at 70 °C after 40 min. In the current method, distilled water was used as the solvent and NQS in potassium hydroxide was used as the chromogenic reagent. The color developed at room temperature after 20 min. Thus, the temperature and time required for the analysis of atomoxetine hydrochloride were found to be minimal in this investigation. Lower LOD and LOQ values established in the current colorimetric method compared with the literature methods further evidence the high sensitivity of the method (Table 4).

CONCLUSION

This nucleophilic addition-based spectrophotometric method developed for the quantification of atomoxetine hydrochloride using NQS in potassium hydroxide was found to be a simple,

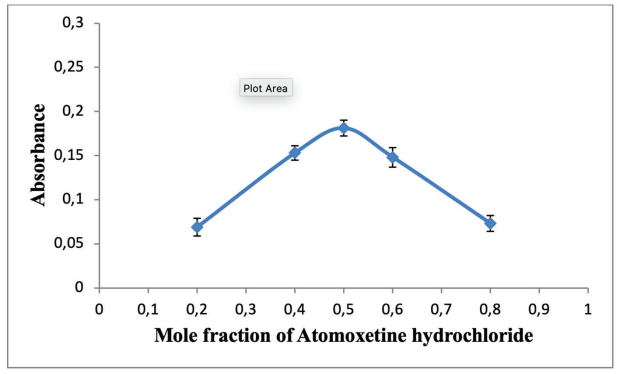

Figure 7. Job's continuous variation plot for the analytical method, X-axis scale as 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1, Y-axis scale as - 0, 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3

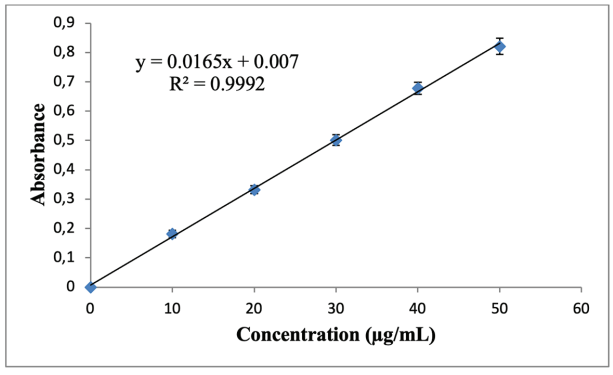

Figure 8. Calibration plot of atomoxetine hydrochloride (10-50 µg/mL) in distilled water, Y-axis scale as - 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9

Table 2. Precision data of the analytical method

Statistical variables	Concentration estimated (µg/mL)[b]	
	Intra-day precision	Inter-day precision
AM ± SD	30.514 ± 0.548	30.646 ± 0.359
% RSD[a]	1.79	1.17

AM: Arithmetic mean, SD: Standard deviation, [a]Acceptance criteria: % RSD should not exceed 2.00, [b]Concentration taken: 30 µg/mL, RSD: Relative standard deviation

Table 3. Assay data of atomoxetine hydrochloride in tablets using the developed method

Formulation	Label claim (mg)	Amount found (mg) (AM ± SD, n: 3)	Bias	% Assay	% RSD*
AXEPTA-10	10	10.152 ± 0.049	0.028	101.52	0.48

AM: Arithmetic mean, SD: Standard deviation, Bias: Standard deviation/square root of sample size.
*Acceptance criteria: % RSD should not exceed 2.00

Table 4. Method conditions in the current and literature methods

S. no.	Method conditions	Validation parameters	References
1	Solvent: Borate buffer and chloroform Reagent: NQS in an alkaline medium, Temperature: 70 °C, Time for development of colour: 40 min, λ_{max}: 449 nm	Rectilinear: 5 - 40 µg/mL LOD: 0.02 µg/mL LOQ: 0.06 µg/mL	4
2	Method A: Solvent: Methanol Reagent: Vanillin in sulphuric acid λ_{max}: 560 nm	Linearity: 1 - 5 µg/mL	5
	Method B: Solvent: Methanol Reagent: *para*-Dimethyl amino benzaldehyde in sulphuric acid λ_{max}: 600 nm	Linearity: 10 - 50 µg/mL	
3	Solvent: Double distilled water, λ_{max}: 270 nm	Linearity: 20 - 180 µg/mL LOD: 4.04 µg/mL LOQ: 12.25 µg/mL	6
4	Solvent: Acetonitrile λ_{max}: 271 nm	Linearity: 20 - 140 µg/mL LOD: 3.5 µg/mL LOQ: 10.62 µg/mL	7
5	Solvent: 0.05 N Hydrochloric acid λ_{max}: 225 nm	Linearity: 5 - 40 µg/mL LOD: 0.154 µg/mL LOQ: 0.467 µg/mL	8
6	Solvent: Distilled water, Reagent: NQS in potassium hydroxide, Temperature: Room temperature, Time for development of colour: 20 min λ_{max}: 474 nm	Linearity: 10 - 50 µg/mL LOD: 0.20 µg/mL LOQ: 0.606 µg/mL	Current method

expeditious, and extraction-free strategy. The use of universal solvents, such as distilled water, makes it a non-pollutant methodology (as no organic solvent was used). Furthermore, a shorter reaction time and analysis at room temperature are more promising features of this method. The proposed analytical method was passed through validation parameters as *per* ICH specification. The % assay was congruent as stated on the label. In addition, mediation of the formulation excipients was found to be nil in the estimation. With these benefits, the suggested methodology can be adopted in routine quality control testing of atomoxetine hydrochloride in its pharmaceutical dosage forms.

Peer-review: Externally and internally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: P.Y., Concept: S.N., D.P., Design: S.N., Data Collection or Processing: P.Y., S.N., Analysis or Interpretation: S.N., P.Y., Literature Search: P.Y., S.C., Writing: S.N., S.C.

Conflict of Interest: No conflict of interest was declared by the authors.

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Supplementary Table 1. Data for the selection of solvent

S. no.	Solvent	Absorbance* (AM ± SD)
1	Distilled water	0.181 ± 0.001
2	Methanol	0.117 ± 0.003
3	Ethanol	0.148 ± 0.006
4	Acetonitrile	0.073 ± 0.004

AM: Arithmetic mean, SD: Standard deviation, *Measured using 10 µg/mL analyte solution