IJBPAS, October, 2024, 13(10): 4958-4969

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ASSESSMENT OF TOTAL ATOMOXETINE CONTENT IN PHARMACEUTICAL FORMULATIONS EMPLOYING MULTIVARIATE CALIBRATION ALGORITHM VIA UV SPECTROPHOTOMETRY

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Received 24th July 2023; Revised 25th Sept. 2023; Accepted 17th Dec. 2023; Available online 1st Oct. 2024

https://doi.org/10.31032/IJBPAS/2024/13.10.8378

INTRODUCTION

The presynaptic reuptake of norepinephrine is selectively inhibited by atomoxetine hydrochloride, or [ATX, (-)-N-methyl-γ-(2-methylphenoxy) benzene propanamine] **(Figure 1)**. An ortho-methyl phenoxy analog of nisoxetine, ATX is a (-) isomer and a phenoxy propylamine analogue. The FDA has approved its first non-stimulant medication for the therapy of attention-deficient hyperactivity disorder (ADHD), Atomoxetine hydrochloride. The most frequently seen neurobehavioral condition in children, ADHD, develops symptoms in certain individuals that persist till adulthood **[1]**. ATX has a high water-soluble content and biological membrane permeability, which enables immediate and complete absorption post oral administration. The activity of CYP2D6 influences ATX oral bioavailability and clearance; nevertheless, plasma pharmacokinetic characteristics are anticipated among extensive and weak metaboliser individuals **[2]**. A specific study's findings also demonstrated significant changes in cognitive levels after taking ATX compared to before the therapy, as well as the ATX group compared to the placebo group **[3]**.

A monograph on Atomoxetine hydrochloride (ATX HCl) can be discovered in the Indian

Pharmacopoeia. The titration procedure with acetous per chloric acid along with acetous mercuric acetate and subsequent potentiometric assessment as the end result has been reported in the Indian Pharmacopoeia (IP) **[4]**. ATX is official in the United States Pharmacopeia (USP) as well as in the British Pharmacopeia (BP).

Figure 1 shows Chemical structure of Atomoxetine.

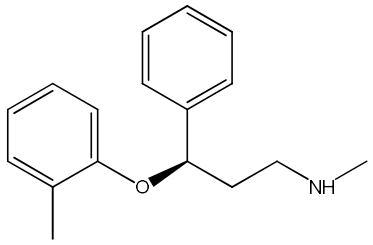

Figure 1: Chemical structure of Atomoxetine

According to the review of the literature, a handful of scientific methods were established in the evaluation of Atomoxetine hydrochloride in pharmaceutical products by employing UV and HPLC, either in isolation **[5]** or simultaneously **[6, 7]** or HPLC solely **[1, 8, 9]**, HPTLC **[10]**, GC-MS **[11]**, LC-MS **[12]**, RP-HPLC **[13]** and spectrofluorimetry **[14]**.

The suggested method provides a higher degree of confidence in results since it directly measures ATX and has been verified with greater accuracy and precision than the standard UV-Visible evaluation. This multi-variant procedure abates the discrete result and transforms to an "m" value as a variable which is reliable. The following formula may be used for any specified wavelength if the absorbance of an analyte (X), i.e. ATX, has been imaged at 5 distinct wavelengths (λ = 266, 268, 270, 272, and 274 nm).

$A_{\lambda 266} = a \text{ x } C_x + k_1$ ---------- (1)

$A_{\lambda 268} = b \text{ x } C_x + k_2$ ---------- (2)

$A_{\lambda 270} = c \text{ x } C_x + k_3$---------- (3)

$A_{\lambda 272} = d \text{ x } C_x + k_4$---------- (4)

$A_{\lambda 274} = e \text{ x } C_x + k_5$---------- (5)

Here A_λ indicates the absorbed energy of the analyte, slopes of the linear regression functions for the analyte are represented as a, b, c, d, e, k1, k2, k3, k4, and k5 are the intercepts at the five specified wavelengths, and C_x constitute the concentration of the analyte. The formula sets (1–5) stated above can be summed together as follows:

$A_T = a \text{ x } C_x + b \text{ x } C_x + c \text{ x } C_x + d \text{ x } C_x + e \text{ x } C_x + K_T$---------- (6)

On further simplifying we get,

$A_T = C_x\,(a + b + c + d + e) + K_T$------ (7)

The total of the intercepts of the regression equations at the specified five wavelengths and the sum of resultant absorbance are represented as A_T and K_T respectively. The equation that follows can be used to figure out the analyte X's concentration.

$$C_x = \frac{A_T - K_T}{(a + b + c + d + e)}$$

According to the requirements for ICH analytical method validation, this approach was created and verified **[15]**.

ANALYTICAL PROCEDURE:

Applied chemicals and solvents:

- Atomoxetine hydrochloride
- The marketed capsule formulation was procured from the local market (Attentrol capsules – 10 mg of Atomoxetine hydrochloride, by Sun Pharma Pvt. Ltd.)
- Distilled water – solvent.

Solubility:

- Sparingly soluble in ethanol, DMSO, dimethyl formamide and Distilled Water.

Instrumentation:

- UV-Vis double beam Spectrophotometer (Lab India UV-3092)
- Microbalance (SHIMADZU BL-220H)
- Ultra Sonic Bath (ILE, ILTC)

METHOD DEVELOPMENT:

Solvent Choice:

Atomoxetine hydrochloride was solubilized in distilled water (30 mg/ml), which was used as the solvent across the analysis.

Standardized stock solution preparation:

Atomoxetine hydrochloride was delicately measured and stored in a 10 mL calibrated volumetric flask. After adding 5 mL solvent, 15 minutes were spent subjecting the mixture to ultra-sonication. With the solvent, the final amount was diluted up to 10 mL (1 mg/mL). 5 mL of the aforementioned preparation was pipetted out and stored in a 50 mL calibrated volumetric flask, utilizing the solvent. The whole volume was brought up to the defined point and thoroughly mixed. The subsequent solution was diluted proportionately to render concentrations extending from 50 - 150 µg/mL employing the solvent.

Estimation of λ_{max}:

Atomoxetine hydrochloride standardized stock solution was diluted with solvent to achieve a concentration of 100µg/mL. The Ultraviolet range of 400-200nm was employed to scan subsequent preparation. The Ultraviolet spectrum of Atomoxetine hydrochloride is depicted in **Figure 2**.

Preparation of sample solution:

The average weight of 20 Atomoxetine hydrochloride capsules (Attentrol capsules, label claim - 10 mg of Atomoxetine hydrochloride) was determined. The capsules were opened, and the contents were brought together and well combined. A weight that equates to 10 mg of Atomoxetine hydrochloride was delicately measured from the merged excipients and solubilized in 5 mL of solvent using sonication for 15 minutes

before being filled to a volume of 10 mL solvent. The aforesaid solution was thoroughly mixed and subjected to filtration. The resulting filtrate was diluted appropriately for subsequent investigation.

PROCEDURE VALIDATION:

The mentioned technique was inspected according to International Council for Harmonisation Q2(R1) protocol to examine specific validation parameters.

SYSTEM EVIDENCE

System validated for perceptivity, perfection, delicacy, linearity according to ICH Q2B guidelines.

Linearity:

Atomoxetine hydrochloride standardized stock solution was diluted proportionately to procure 50, 75, 100, 125, and 150 µg/mL concentrations. To provide linear correlation and exclude instrumental fluctuations (16,17), around the drug's maximum emission wavelength (270 nm), five additional wavelengths were chosen in order to measure the resulting concentration's absorbance: 266, 268, 270, 272, and 274 nm **(Table 1)**. **Figure 3** displays an overlaid UV spectra establishing linearity. The established logistic regression algorithms for five wavelengths' specific correlation coefficient figures have been derived **(Table 2)**. **Figure 3** depicts the calibration graphs created at five different wavelengths.

Detection Limit (LOD) and Quantification limit (LOQ):

The designed approach was assessed by detecting lowest limit of Detection (LOD) and lowest limit of Quantification (LOQ) values employing the intercept as well as slope values of the logistic regression line.

Precision:

By measuring the absorbed energy of the linearity mixture at 100% concentration (100µg/mL) across all 5 wavelengths, intra-day and inter-day precision measurements were performed. A total of six occasions in a day (intra-day precision) and three occasions a day (inter-day precision) were used to scan the chosen concentration. **Figure 4** displays the overlaid UV spectra for inter and intraday precision assessments, while **Tables 3-6** give the absorbed energy values procured at the designated wavelengths for intra-day and inter-day precision studies. Standard deviation (SD) and percentage relative standard deviation (% RSD) numbers were computed.

Assay:

The absorbed energy of sample mixture was recorded at 270nm. The assay observations are presented in **Table 7** along with a

calculation of the drug content of the formulation.

Accuracy (Recovery studies):

At concentration levels of 50%, 100%, and 150%, recovery tests using the typical addition technique were performed to evaluate the novel methodology's accuracy. 0.4 mL of the sample solution was pipetted into three different 10 mL volumetric flasks from the sample stock solutions, and 0.1, 0.6, and 1.1 mL of the standard stock solution were pipetted into the same volumetric flask. The remainder was made up of distilled water. The recovery % values were calculated. **Figure 5** shows the overlay UV spectra, and **Table 8** lists the recovery investigations' conclusions.

OUTCOMES AND DISCUSSION:

Atomoxetine hydrochloride's absorption maxima were noted at 270 nm.

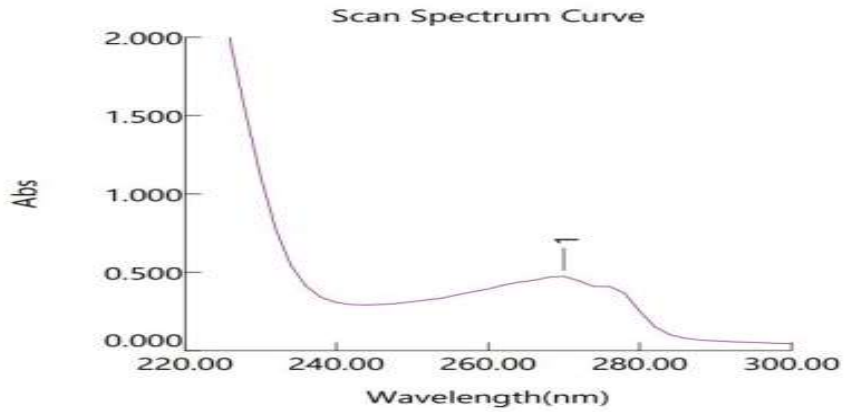

Figure 2: UV Spectra of Atomoxetine Hydrochloride

Linearity:

Across a certain concentration range of 50 to 150µg/mL, the designed approach appears linear. Linear regression equation was constructed for all the five selected wavelengths of 266, 268, 270, 272 and 274 nm. The obtained correlation coefficient values are all greater than 0.998.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The calculated LOD and LOQ values range from 1.18 to 1.82 µg/mL and from 3.59 to 5.52 µg/mL, respectively.

Precision:

The percentage Relative Standard Deviation figures for intra-day and inter-day precision were observed to be much lower than the ICH acceptability standard of 2%, falling in the range of 0.08-0.78 and 0.19-0.29, respectively. The precise nature of the

established methodology is demonstrated by the low estimated % RSD figure.

Assay:

The absorbed energy of the sample mixture was recorded at 270nm and the aggregate of Atomoxetine hydrochloride present in the capsule formulation was studied. The percentage of assay experiments was 99.90% w/w and the anticipated RSD% turned out to be below 2%.

Recovery:

The drug's percentage recovery was computed and determined to be between 99.35 and 102.9% w/w, which was determined to fall within the ICH protocol's limit between 97 to 103% w/w. Consequently, the procedure can be deemed accurate.

CONCLUSION:

For the assessment of Atomoxetine hydrochloride in capsule formulation, the designed simple and quick assessment of total Atomoxetine content in pharmaceutical formulations employing multivariate calibration algorithm via UV spectrophotometry was found to be linear, sensitive, accurate, and precise. The medication's absorbed energy is determined at 5 distinct designated wavelengths; therefore, the multivariate calibration methodology established was more reliable than prior published methodologies. The current created approach for quantifying Atomoxetine hydrochloride was compared to previously published methods, and the current method was shown to be highly sensitive, with low stated LOD and LOQ values. All validation parameters evaluated were found to be within the limits indicated by ICH specifications. The established approach may be expanded to quantify Atomoxetine hydrochloride in distinct pharmacological dosage forms like injectables, tablets, and inhalation powders. As a result, a simple and quick approach based on statistical concepts was established, and it was found to be more reliable than functioning spectrophotometric methods. This method is now strongly encouraged for application in conventional evaluations of the quality of Atomoxetine hydrochloride in drug formulations.

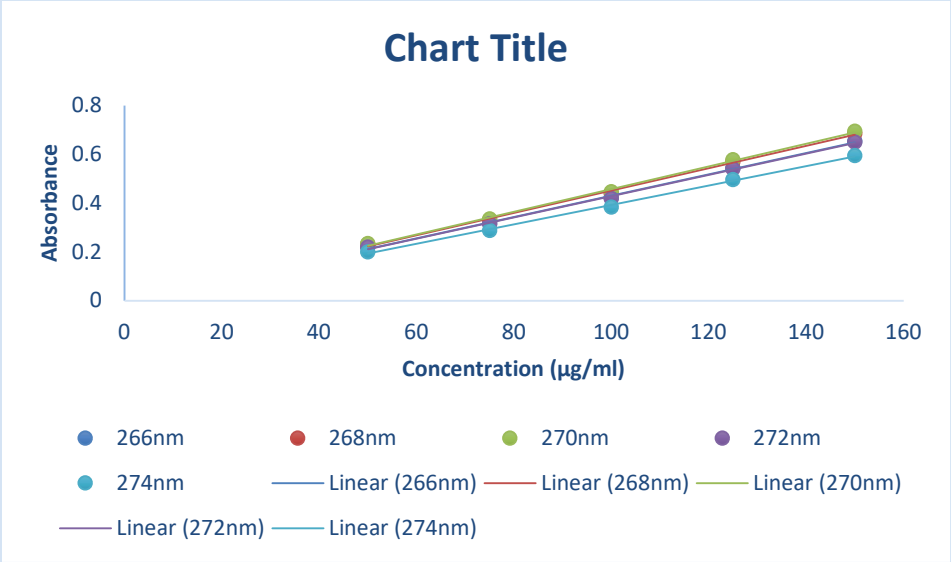

Figure 3: Calibration graph at five selected wavelengths

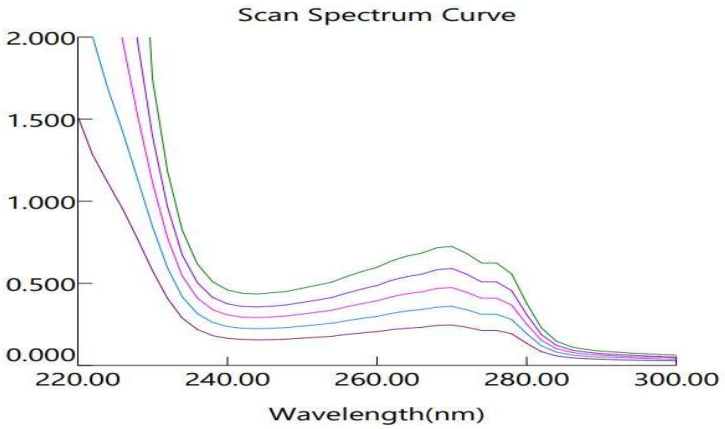

Figure 3 A: UV Spectrum of Atomoxetine Hydrochloride showing linearity 314nm.

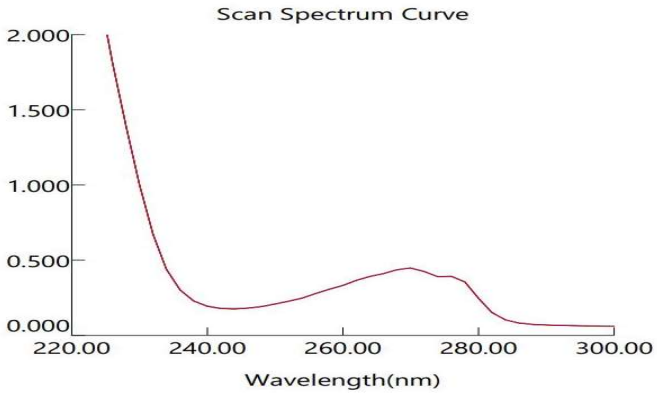

Figure 4: Overlay UV Spectra of ATX HCl showing inter and intraday precision studies

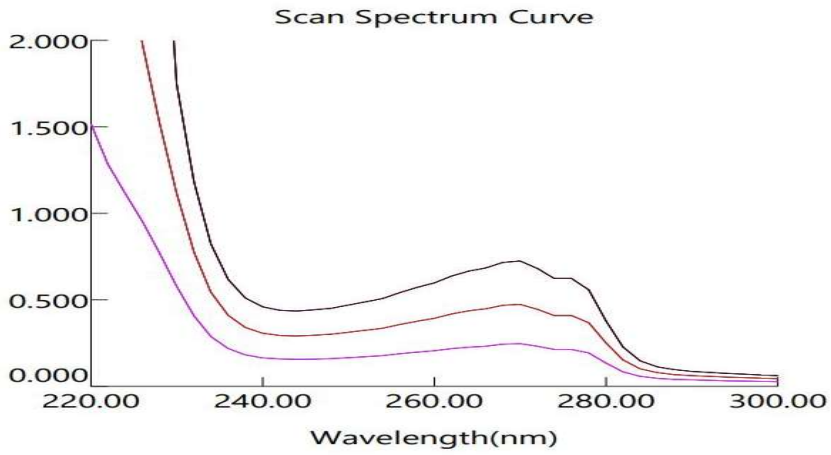

Figure 5: Overlay UV Spectra of Atomoxetine Hydrochloride showing accuracy

Table 1: Multivariate UV calibration at five selected wavelengths

Concentration (µg/mL)	Absorbance (nm)				
	266	268	270	272	274
50	0.220	0.231	0.234	0.219	0.200
75	0.316	0.331	0.335	0.315	0.288
100	0.421	0.440	0.446	0.419	0.383
125	0.543	0.569	0.577	0.541	0.496
150	0.653	0.685	0.694	0.650	0.594

Table 2: Linearity data showing system suitability parameters at the selected wavelengths

Wavelength (nm)	Regression equation	r^2	Average of Slope	SD of Intercept	LOD (µg/mL)	LOQ (µg/mL)
266	y = 0.0034x + 0.0034	0.999	0.0038	0.0021	1.82	5.52
	y = 0.0037x + 0.00484	0.999				
	y = 0.0035x + 0.0036	0.9995				
	y = 0.004x + 0.00834	0.9998				
	y = 0.0044x - 0.0066	0.9983				
268	y = 0.0046x - 0.0072	0.998	0.0044	0.00179	1.31	3.99
	y = 0.0046x - 0.0092	0.998				
	y = 0.0044x - 0.0076	0.999				
	y = 0.0044x - 0.0056	0.998				
	y = 0.0044x - 0.0046	0.998				
270	y = 0.0048x + 0.0022	0.998	0.0046	0.00241	1.70	5.16
	y = 0.0046x - 0.0076	0.998				
	y = 0.0046x - 0.0076	0.998				
	y = 0.0047x - 0.0056	0.998				
	y = 0.0046x - 0.0036	0.998				
272	y = 0.0044x - 0.0084	0.998	0.0044	0.00158	1.18	3.59
	y = 0.0044x - 0.0074	0.998				
	y = 0.0044x - 0.0064	0.998				
	y = 0.0044x - 0.0054	0.998				
	y = 0.0044x - 0.0044	0.998				
274	y = 0.004x - 0.0082	0.998	0.004	0.00158	1.30	3.95
	y = 0.004x - 0.0072	0.998				
	y = 0.004x - 0.0062	0.998				
	y = 0.004x - 0.0052	0.998				
	y = 0.004x - 0.0042	0.998				

Table 3: Absorbance values for intraday precision studies

Concentration (µg/mL)	Number of repetitions	Absorbance				
		266 nm	268 nm	270 nm	272 nm	274 nm
100	1	0.412	0.437	0.449	0.426	0.391
	2	0.411	0.437	0.448	0.425	0.391
	3	0.411	0.436	0.447	0.424	0.390
	4	0.409	0.435	0.447	0.423	0.390
	5	0.410	0.436	0.446	0.423	0.390
	6	0.410	0.435	0.446	0.423	0.389

Table 4: Absorbance values for interday precision studies

Concentration (µg/mL)	Number of repetitions	Absorbance				
		266 nm	268 nm	270 nm	272 nm	274 nm
50	1	0.206	0.219	0.225	0.213	0.196
	2	0.205	0.219	0.225	0.212	0.195
	3	0.206	0.218	0.224	0.211	0.193
100	1	0.449	0.468	0.474	0.445	0.409
	2	0.448	0.467	0.474	0.444	0.408
	3	0.448	0.468	0.473	0.442	0.407
150	1	0.619	0.657	0.674	0.639	0.589
	2	0.618	0.656	0.671	0.638	0.588
	3	0.618	0.656	0.674	0.637	0.587

Table 5: Interday precision study

Concentration (µg/mL)	Description	266 nm	268 nm	270 nm	272 nm	274 nm
50	Mean	0.20566667	0.21866667	0.22466667	0.212	0.19466667
	SD	0.00057735	0.00057735	0.00057735	0.001	0.00152753
	%RSD	0.28	0.26	0.25	0.47	0.78
100	Mean	0.44833333	0.46766667	0.47366667	0.44366667	0.408
	SD	0.00057735	0.00057735	0.00057735	0.00152753	0.001
	%RSD	0.12	0.12	0.12	0.34	0.24
150	Mean	0.61833333	0.65633333	0.673	0.638	0.588
	SD	0.00057735	0.00057735	0.00173205	0.001	0.001
	%RSD	0.09	0.08	0.25	0.15	0.17

Table 6: Intraday precision study

Concentration (µg/mL)	Description	266 nm	268 nm	270 nm	272 nm	274 nm
100	Mean	0.4105	0.436	0.447167	0.424	0.390167
	SD	0.001049	0.000894	0.001169	0.001265	0.000753
	% RSD	0.25	0.20	0.26	0.29	0.19

Table 7: Assay of Atomoxetine Hydrochloride in marketed pharmaceutical formulation

Label claim (mg)	Amount estimated (mg)	%Assay
10.0	9.98	99.80
	10.01	100.10
	9.97	99.70
	10.03	100.3
	9.96	99.6
Average		99.90
SD		0.2608
% RSD		0.26

Table 8: Recovery studies

Wavelength (nm)	Conc. levels (%)	Sample Conc. Present (µg/mL)	Standard Conc. (µg/mL)	Final Conc. (µg/mL)	Amount recovered (µg/mL)	% Recovery
266	50	40	10	50	9.84	99.46
	100	40	60	100	60.15	100.25
	150	40	110	150	109.65	99.61
268	50	40	10	50	9.97	99.90
	100	40	60	100	59.98	99.96
	150	40	110	150	109.76	99.73
270	50	40	10	50	10.89	102.9
	100	40	60	100	59.89	99.81
	150	40	110	150	110.91	101.01
272	50	40	10	50	29.97	99.9
	100	40	60	100	59.61	99.35
	150	40	110	150	110.97	101.07
274	50	40	10	50	9.82	99.40
	100	40	60	100	59.99	99.98
	150	40	110	150	109.96	99.95

ETHICAL STATEMENT

There are no subjects who are humans or any other living creatures used in this study's trials.

ACKNOWLEDGMENTS

The Authors acknowledge SRM College of Pharmacy and the management of SRM Institute of Science and Technology for their support for providing extensive reprographic material for this work.

A DISAGREEMENT OF INTEREST

The authors report no conflicts of interest on the study**.**

FINANCIAL SOURCES

No backing has been reported.

REFERENCES:

[1] Dogrukol-Ak, D., & Yeniceli, D. (2010). A simple and specific HPLC method for the determination of atomoxetine in pharmaceuticals and human plasma. *Journal of liquid chromatography & related technologies*, *33*(19), 1745-1759.

[2] Sauer, J. M., Ring, B. J., & Witcher, J. W. (2005). Clinical pharmacokinetics of atomoxetine. *Clinical pharmacokinetics*, *44*, 571-590.

[3] Nejad, E. M., Amouzadeh, E., Kashipazha, D., Shamsaei, G., & Cheraghian, B. (2023). The effect of atomoxetine on cognitive function in patients with multiple sclerosis.

[4] Naraparaju, S., Yamjala, P., Chaganti, S., Anumolu, D. P., & Naraparaju, S. (2023). Spectrophotometric quantification of Atomoxetine hydrochloride based on nucleophilic substitution reaction with NQS. *Turkish Journal of Pharmaceutical Sciences*.

[5] Koradia, S. K., Shah, P. T., Rana, R. R., Vaghani, S. S., Pandey, S., & Jivani, N. P. (2009). Spectrophotometric determination of atomoxetine hydrochloride from its pharmaceutical dosage forms. *Asian Journal of Research in Chemistry*, *2*(3), 258-259.

[6] Guo, W., 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. *Journal of Chromatography B*, *854*(1-2), 128-134.

[7] Faridi, N., Ghasemi, N., Qomi, M., & Ramezani, M. (2019). Prediction of the Trace Amounts of Atomoxetine in Biological Samples using optimized solvent bar microextraction technique coupled with HPLC-UV. *Journal of Applied Chemical Research*, *13*(4), 28-41.

[8] Rahman, Z., Aanandhi, V., & Sumithra, M. (2018). Analytical method development and validation of atomoxetine hydrochloride using rapid high-performance liquid chromatographic technique. *Asian Journal of Pharmaceutical and Clinical Research*, 118-120.

[9] Prajapati, H. R., Raveshiya, P. N., & Prajapati, J. M. (2011). RP-HPLC determination of atomoxetine hydrochloride in bulk and pharmaceutical formulations. *Journal of Chemistry*, *8*, 1958-1964.

[10] Prajapati, H. R., Raveshiya, P. N., & Prajapati, J. M. (2011). RP-HPLC determination of atomoxetine hydrochloride in bulk and pharmaceutical formulations. *Journal of Chemistry*, *8*, 1958-1964.

[11] Kul, A., Ozdemir, M., Önal, A., & Sagirli, O. (2021). A novel GC–MS assay method for the therapeutic drug monitoring of the atomoxetine. *Microchemical Journal*, *164*, 105953.

[12] Xia, Y., Guo, H. L., Hu, Y. H., Long, J. Y., Chen, J., Chen, F., & Ji, X. (2021). Determination of atomoxetine levels in human plasma using LC-MS/MS and clinical application to Chinese children with ADHD based on CPIC guidelines. *Analytical Methods*, *13*(21), 2434-2441.

[13] Ravisankar*1 P, , Kamma Harshasri PSB. Development and Validation of RP-HPLC Method for the Determination of Atomoxetine Capsules. IOSR J Pharm. 2019;9(5):16–27.

[14] Ulu, S. T. (2011). Spectrophotometric and spectrofluorimetric determination of atomoxetine in pharmaceutical preparations. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, *66*(11), 831-835.

[15] The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH Harmonised TRIPARTITE Guideline Validation OF Analytical Procedures : Parent Guideline : Text on Validation of Analytical Procedures. 2005;1994(November).

[16] Kathirvel, S., Sahiti, V., & Suneetha, A. (2010). Spectrophotometric Estimation of Risedronate Sodium in Bulk and Pharmaceutical Formulations using Multivariate Technique. *Research Journal of Pharmacy and Technology*, *3*(4), 1209-1212.

[17] Karthick, R. K., Kavitha, J., & Lakshmi, K. S. (2020). Quantification of Terbutaline Sulphate in Pharmaceutical Formulation by UV Spectrophotometric aided Multivariate Calibration Technique. *Research journal of Pharmacy and Technology*, *13*(2), 767-773.