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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF METOLAZONE

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Abstract:

A cost-effective and unique RP-HPLC technology has been developed to correctly and precisely quantify the amount of Metolazone in both bulk amounts and solid dosage forms. The separation procedure was performed with an Inertsil ODS-3 HPLC Column with a particle size of 5 µm and dimensions of 250 x 4.6 mm. The experiment used the isocratic method, with a mobile phase composed of an 80:20 (v/v) blend of Acetonitrile and methanol. A pump was used to introduce the mobile phase into the column at a flow rate of 1.0 mL min-1. The eluent from the column was identified using a UV detector configured to function at a wavelength of 295 nm. The experiment lasted for 8 minutes, during which the column was continually maintained at the surrounding temperature. The calculated retention time for Metolazone was 3.557 minutes. The standard curves demonstrated a consistent linear correlation over the concentration range of 02-10 µg/ml, with a very high coefficient of determination (R2 = 0.9997). The investigation yielded a range of percentage recoveries, spanning from 98% to 102%. In addition, the Relative Standard Deviation (RSD) was calculated to be 0.290%. The percentage content of a commercially available version of Metolazone was tested to be 100.18%. The technique was validated in compliance with the guidelines established by the International Council for Harmonisation of Technical guidelines for Pharmaceuticals for Human Use (ICH). The proposed RP-HPLC technology has undergone validation via study, demonstrating its attributes of simplicity, specificity, speed, reliability, and consistency. Hence, the methodology proposed in this research may be used for the routine examination of Metolazone in both its concentrated and solid forms, particularly with the objective of ensuring quality assurance.

Keywords: Metolazone, Method development, Method validation and RP-HPLC

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Introduction:

Atomoxetine, often known as ATOX, is the first medicine that does not include stimulants that has been approved for the treatment of attention-deficit hyperactivity disorder (ADHD). The Hydrochloride salt of Atomoxetine is the name that is used to promote this medicine. It acts as a noradrenaline inhibitor that is unique to the substance. [1] The molecule is referred to by its IUPAC name, which is (R)-N-methyl-3-phenyl-3-(o-tolyloxy) propan-1-amine at the moment. The medication known as atomoxetine is classified as a norepinephrine reuptake inhibitor, and it has been approved for usage in people of all ages, including children, adolescents, and senior citizens. In spite of this, there has been no investigation on whether or not the therapy is successful in children less than six years old. When compared to stimulants, this medicine used to treat attention-deficit/hyperactivity disorder (ADHD) has a lesser risk for addiction. It is also not considered a limited medication, and it has been shown in clinical research to give continuous symptom alleviation for a period of twenty-four hours in both adults and children who suffer from attention-deficit/hyperactivity disorder [1].

Strattera was first created as a revolutionary antidepressant medicine; however, research conducted in clinical settings did not reveal any therapeutic benefits associated with the prescription. Based on the hypothesis that norepinephrine plays a role in attention-deficit/hyperactivity disorder (ADHD), Strattera was successfully tested and approved for use as a treatment for the illness. Atomoxetine is now being tested in clinical studies to see whether or not it is effective in weight loss programs for those who are obese or who have a problem with binge eating.

Analysis of the chemical atomoxetine was performed using the high-performance liquid chromatography (HPLC) technique described in the United States Pharmacopoeia. This strategy makes use of a mobile phase in conjunction with a gradient flow technique, which is notable for being difficult, time-consuming, and complex [2]. The examination of atomoxetine HCl has been recorded using a wide variety of methods, including ultraviolet (UV), high-performance liquid chromatography (HPLC), high-performance liquid chromatography (HPLC-MS), and high-performance thin-layer chromatography (HPTLC) [3-18]. The strategy that has been proposed is intended to be effective in terms of both time and chemical consumption. More specifically, it seeks to lessen the quantity of chemicals that are used in the mobile phase, solvent, and the medicine that is being investigated. When it comes to producing exact estimates of the concentrations of atomoxetine HCL in both bulk and prescription dosage forms, it also makes an effort to deliver correct findings and to be as clear as possible.

Materials & Method:

HPLC grade water and methanol were purchased from Merck chemicals, Mumbai.

The HPLC system used in this study was a Perkin Elmer instrument from the United States. It included a Binary LC Pump 200 (Perkin Elmer, Model: series 200), a vacuum degasser, and a UV-VIS detector (Perkin Elmer, Model: series 200) with Rheodyne sample injector system equipped with a 20µl sample loop and Total Chrome Navigator software, namely version V 6.3.1. The vacuum filtration assembly from Orchid Scientifics, namely the JVP01 model. Additionally, an ultrasonicator from Oscar, specifically the Microclean 103 model.

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The separation technique was conducted using an Xterra RP 18 column, which had dimensions of 250 mm × 4.6 mm and a particle size of 5 µ. The mobile phase consisted of a mixture of water and methanol at a proportion of 70:30. An injection volume of 20 µL was used for the analysis of the samples. The flow rate remained consistent at 1.0 mL/min for a duration of 5 minutes. The temperature was accurately maintained at a constant 25°C during the whole analytical process. The pharmaceutical substances were identified and their purity was evaluated using a UV detector set to operate at a wavelength of 280 nm.

Preparation of standard solution:

Precisely measured 10 mg of Metolazone standard and placed it into a 10 ml volumetric flask. The substance was dissolved in a specific quantity of water and vigorously mixed until fully dissolved. The volume was then adjusted using the same solvent to achieve a final concentration of 1000 µg/ml (standard stock solution A). Using the stock solution mentioned above, a 1 ml portion was transferred into a 10 ml volumetric flask. Water was added to fill the flask up to the mark, resulting in a final concentration of 100 µg/ml (referred to as standard stock solution B).

Preparation of sample solution:

Precisely transfer a quantity equivalent to 10 milligrams of Metolazone into a 100 milliliter volumetric flask, ensuring that no tablet powder is lost throughout the process. To prepare the solution, combine 60 ml of water and vigorously stir to facilitate dissolution. Subsequently, achieve the desired concentration of the solution by adding more diluent and ensuring thorough homogenization. 1 mL of the filtrate was extracted and then transferred to a 10 mL volumetric flask. The flask was then filled with a diluent until it reached its maximum capacity of 10 mL.

The optimized chromatographic conditions:

The experiment was conducted using an Xterra RP 18 column with dimensions of 250 mm × 4.6 mm and a particle size of 5 µ. The flow rate was set at 1.0 ml/min, and a detection wavelength of 280 nm was utilized. The column temperature was kept at ambient conditions. The injection volume was 20 µl, and the experiment ran for a duration of 5 minutes. Figure 01 displays a representative chromatogram.

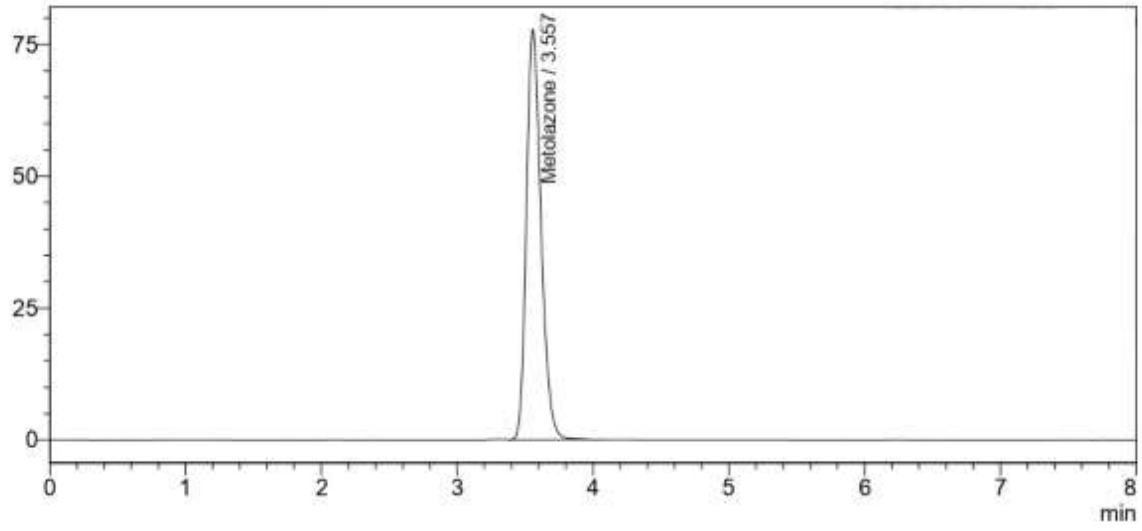

Fig.No. 01: Typical Chromatogram of Standard Metolazone

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Validation of Proposed method:

The developed technique underwent validation following the criteria outlined by the International Conference on Harmonization (ICH). The validation method included several elements such as system suitability, accuracy, specificity, forced degradation studies, linearity, precision, limit of detection, and limit of quantification.

Validation Parameters:

System Suitability:

To optimize the performance of the analytical system, it is essential to determine the parameters that are most appropriate for the system. The established procedure included quantifying about 10 mg of Metolazone, thereafter transferring it into a 10 ml volumetric flask. Afterward, 5 ml of diluent was added to aid in dissolving the substance, and the resulting mixture was vigorously agitated for about 10 minutes. Ultimately, the solution's volume was modified to the intended amount by including more diluent. In addition, the solution was diluted to achieve a concentration of 05 µg/ml. The findings indicate that the relative standard deviation (% RSD) is below 2.0%, the plate count exceeds 8000, and the peak symmetry is below 1.2. The findings are recorded and organized in Table 1.

Table No. 01: System Suitability Data

Injection Number	Retention Time	Peak Area	Plate Count	Peak Symmetry
1	3.05	1178937	10112	1.02
2	3.08	1175648	11235	1.05
3	3.1	1178561	10456	1.09
4	3.11	1172389	10756	1.1
5	3.06	1175614	10589	1.11
6	3.07	1173698	10568	1.05
Average	3.08	1175808		
Standard Deviation	0.02	2591.31		
% RSD	0.7526	0.2204		

Linearity:

Samples of 2, 4, 6, 8, and 10 ml were taken from a solution containing Metolazone at a concentration of 100 µg/ml. The aliquots were then diluted with a diluent until the desired volume was attained. The concentrations obtained for Metolazone ranged from 02 to 10 parts per million (ppm). We performed triplicate injections of each sample at every concentration level, using a 20 µl volume for each injection. Subsequently, a calibration curve was generated by graphing the peak area against the corresponding drug concentration. A direct correlation was seen between the peak area and concentration within the range under investigation. Figure 2 depicted the measurements and calibration curve, whilst Table 1 included the findings.

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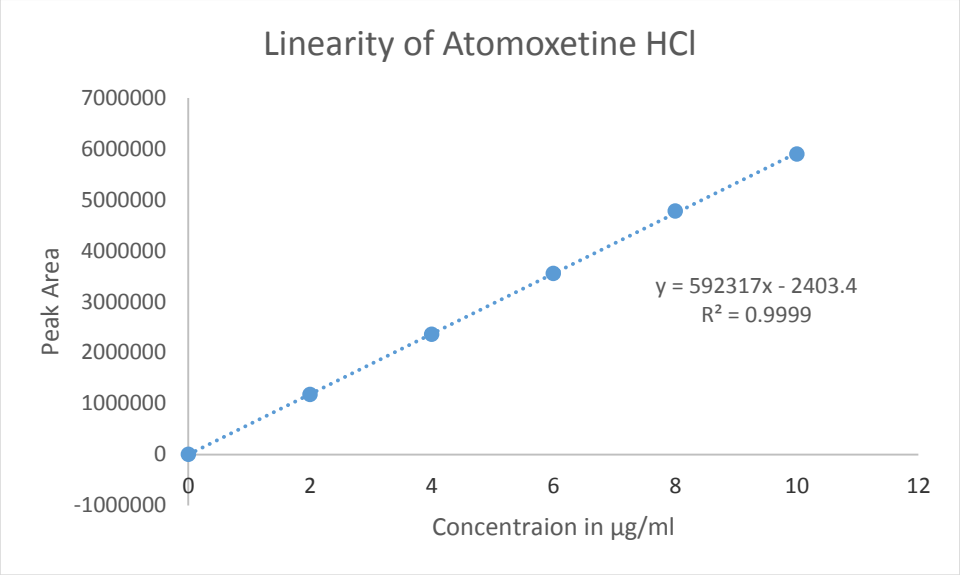

Fig.No. 02: Linearity Plot of Metolazone

Table 2: Linearity data

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	2	1178937
3	4	2354782
4	6	3549856
5	8	4776842
6	10	5894687
Slope		592317
Intercept		-2403.4
Regression		0.9999

Precision:

10 milligrams of Metolazone were precisely measured and added to a 100 milliliter volumetric flask. Afterward, 50 milliliters of an appropriate diluent were introduced into the flask to aid in the process of dissolving. The mixture was let to dissolve for a period of 10 minutes. The capacity was then increased to 100 ml with the use of a diluent. The solution was further diluted to obtain a concentration of 25 µg/ml. The conventional approach included creating six replicas of the solution, and the resultant data was documented in a tabular style in Table No.03.

Table 3: Precision data

Sample Preparation No.	Assay (%)
1	100.45

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2	99.95
3	99.65
4	100.23
5	100.24
6	100.56
Mean	100.18
SD	0.33
RSD (%)	0.3332

Accuracy:

In order to assess the accuracy of the suggested method, recovery experiments were conducted at three different levels: 80%, 100%, and 120% of the target concentration, adhering to the guidelines set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). The recovery inquiry was conducted in duplicate for every level. The findings of the recovery investigations are shown in Table 4.

Table 4.Recovery Studies

Level	Amount found (mg/ml)	Amount added (mg/ml)	Recovery (%)	Mean (%)
Level-1 (80%)	4.01	4	100.25	100.75
	4.03	4	100.75	
	4.05	4	101.25	
Level-2 (100%)	5.01	5	100.2	100.53
	5.03	5	100.6	
	5.04	5	100.8	
Level-3 (120%)	6.01	6	100.17	100.44
	6.02	6	100.33	
	6.05	6	100.83	
Mean			100.49	
SD			0.30	
% RSD			0.2950	

Robustness of method:

A research was conducted to determine the impact of deliberate modifications in the ideal chromatographic settings. The criteria include the mobile phase composition, flow rate, and wavelength. An inquiry was carried out to ascertain the impact of these modifications on the

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system's suitable metrics, such as the tailing factor and the number of theoretical plates, as well as the percentage of relative standard deviation (RSD). Only one prerequisite was modified, while all other conditions remained same. This was done to ensure the preservation of uniformity. It was determined that the acquired findings fell within the permitted thresholds, indicating the procedure's specificity. The outcomes of the study undertaken to ascertain the experiment's dependability are shown in Table 5.

Table 5: Method Robustness

Condition	% RSD	Tailing Factor	% Recovery	No. of Theoretical plates
1**) Change in Flow rate**				
Normal Condition (1.0ml per minute)	0.39	1.01	99.52	10456
Flow rate(1.2 ml per minute)	0.48	1.05	99.56	10235
Flow rate(0.8 ml per minute)	0.65	1.08	100.38	10458
2) Change in minor component in the mobile phase				
Normal Condition (Water : Methanol) (70 : 30))	0.68	1.09	99.12	10238
(Water : Methanol) (60 : 40))	0.43	1.05	99.89	10367
(Water : Methanol) (80 : 20))	0.72	1.01	100.12	10562
3) Change in Wave Length				
Normal: Wave Length 280 nm	0.86	1.05	99.87	10238
Wave Length 285 nm	0.46	1.06	99.82	10751
Wave Length 275 nm	0.28	1.01	100.46	10645

Ruggedness:

A ruggedness test was conducted to assess and compare the performance of two distinct analysts, equipment, and columns. The analytical approach that was developed may be considered trustworthy based on the fact that the coefficient of determination (RSD) value was below 2.0%. The corresponding results are shown in Table 6.

Table 6. Method Ruggedness

Sample Preparation No.	Assay (%)
1	100.25
2	100.56
3	100.42
4	99.56
5	99.12

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6	99.87
Mean	99.96
SD	0.55
RSD (%)	0.5533
Difference between method precision and intermediate precision assay	**0.22**

Conclusion:

The primary objective of this work was to establish a sensitive and accurate high-performance liquid chromatography (HPLC) technique for the analysis of Metolazone in both its pure drug state and pharmaceutical formulations. The experiment used a flow rate of 1.0 ml/min with the Xterra RP C18 column. The mobile phase consisted of a mixture of HPLC grade water and methanol at a volumetric ratio of 70:30. The measured wavelength was 280 nm. The experimental setup involved the utilization of a Perkin Elmer High-Performance Liquid Chromatography (HPLC) system, which consisted of a Binary LC Pump 200 (Perkin Elmer, Model: series 200), a vacuum degasser, and a UV-VIS detector (Perkin Elmer, Model: series 200). The system also included a Rheodyne sample injector system equipped with a 20µl sample loop and Total Chrome Navigator software. The retention times were determined to be 3.08 minutes. The analytical technique underwent validation in accordance with the criteria established by the International Council for Harmonisation of Technical guidelines for Pharmaceuticals for Human Use (ICH), specifically ICH Q2b. The study yielded a correlation coefficient (r2) of 0.9999, indicating a very robust positive connection between the variables. The recovery percentage ranged from 99% to 101%, demonstrating a notable level of accuracy in the measurement technique. The precision of the results, as determined by repeating the injection, was evaluated using the relative standard deviation (RSD), which was measured to be 0.2950. This score indicates a considerable degree of variability in the data. The accuracy study showed a remarkable level of precision, durability, and uniformity.

The experiment focused on evaluating the system's appropriate characteristics by using six replicas of a standardized medication solution. The projected values were deemed to satisfy the predetermined acceptance criteria. The tailing factor, number of theoretical plates, and height equivalent to a theoretical plate (HETP) meet the permissible criteria. Therefore, the author asserts that the HPLC method suggested in this study exhibits both sensitivity and consistency when used to measure the amount of Metolazone in pharmaceutical products. Moreover, the approach has a fast analysis time.

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