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Development and Validation of Spectrophotometric Methods for the Assay of Atomoxetine Hydrochloride in Pharmaceutical Preparations

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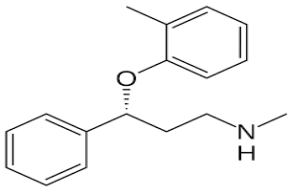
ABSTRACT

Atomoxetine hydrochloride is the medicine for the treatment of an ADHD (Attention deficit hyperactivity disorder) approved by the Food and Drug Administration (FDA). *In this study, a new, precise simple, reliable and sensitive UV spectrophotometric method was developed and validated for the Atomoxetine Hydrochloride in bulk and in Pharmaceutical dosage forms. Atomoxetine Hydrochloride was estimated at 520-740 nm using 0.1 M Hydrochloric acid. An attempt is hereby made to develop simple spectro photometric method in 0.1 M Hydrochloric acid for its direct applicability in dissolution and bioavailability studies of drug in solid dosage forms. The drug obeyed the Beer's law in the range of 04-25 µg mL^{-1} and showed correlation coefficient 0.9999 at 585 nm. The results of analysis were validated by recovery studies. The % recovery was found to be 99.09 – 99.59%. The method was found to be simple, accurate, precise, economical, reliable and reproducible.*

Keywords: Development, Validation, Assay of Atomoxetine Hydrochloride.

INTRODUCTION

Atomoxetine [1] is designated chemically as (-)-*N*-methyl-3-phenyl-3-(o-tolyloxy)-propylamine hydrochloride, is a drug approved for the treatment of attention-deficit hyperactivity disorder (ADHD).



Atomoxetine

It is sold in the form of the hydrochloride salt of atomoxetine, a norepinephrine reuptake inhibitor. Atomoxetine is a white solid that exists as a granular powder inside the capsule, along with pre-gelatinized starch and dimethicone. This compound is manufactured, marketed and sold in the United States under the brand name Strattera by Eli Lilly and Company.

Several assay techniques have been described for quantitative determination of ATXT in biological fluids. These include procedures based on high performance liquid chromatography (HPLC) [2-7], fluorometry [8], and radioimmunoassay [9-10] and gas chromatography [11]. A few reports deal with the analysis of the drug in dosage forms, such procedures include: RP-HPLC [12] in biological fluids. Hence, there is a need for sensitive, rapid reliable method for the routine analysis of ATXT in bulk and pharmaceutical dosage forms. An attempt has been made by the author by exploiting the functional groups present in the drug with suitable reagents and succeed in developing five visible spectrophotometric methods for the assay ATXT in bulk and pharmaceutical dosage forms. In contrast with previous methods; the developed five methods have many advantages. Firstly, it does not need expensive apparatus. Secondly, it is simple and rapid. Thirdly, its linear range is relatively wide. Fourthly, it has good selectivity and high sensitivity.

MATERIALS AND METHODS

Instruments Used: Shimadzu UV-Visible double beam Spectrophotometer (model 1601) was used for spectral studies. Shimadzu - UV– 150-20 was used in validation parameter. An Elico LI-120 digital pH meter was used for pH measurements.

Preparation of standard drug solutions for methods {M_1, M_2, M_3, M_4 & M_5}:
Stock solution (1mg mL^{-1}) of ATXT was prepared by dissolving 100mg of it to 100mL with distilled water. Working standard solution of ATXT were prepared from stock solution by appropriate dilution with distilled water to attain corresponding concentrations 250 $\mu g.mL^{-1}$ [M_1], 100 $\mu g.mL^{-1}$ [M_1 & M_2], 80 $\mu g.mL^{-1}$ [M_1] and 50 $\mu g.mL^{-1}$ [M_3]. All the chemicals and reagents used were of analytical grade and solutions were prepared in doubly distilled water.

Method M_1:

MBTH Solution (Aldrich; 0.2%, 8.56 x 10^{-3}M)	Prepared by dissolving 200mg of MBTH in 100mL of distilled water.
$NaIO_4$ solution (BDH; 0.2%, 9.35 x 10^{-3}M)	Prepared by dissolving 200mg of sodium meta periodate in 100mL of distilled water and standardized iodometrically.

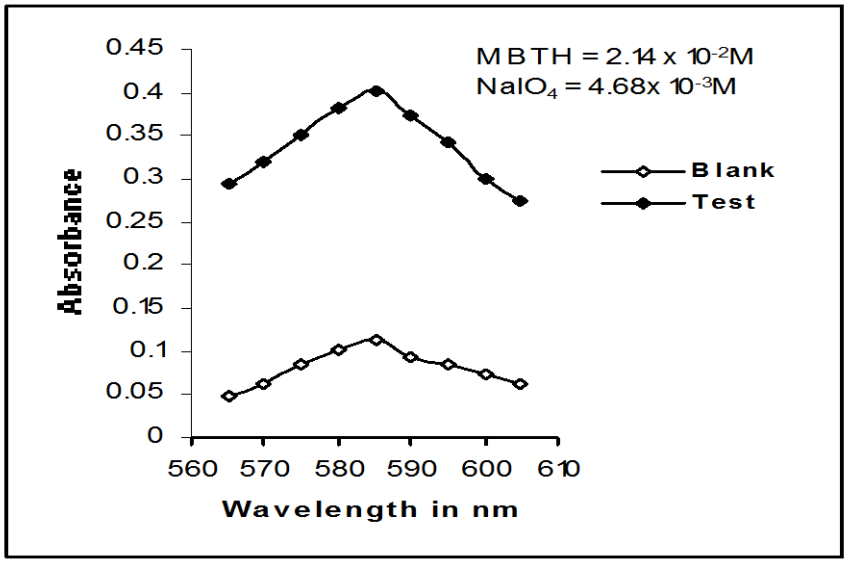


Fig. 1: Absorption spec of ATXT with MBTH - $NaIO_4$ (M_{10})

Method M_2:

MBTH solution (Loba, 0.5%, 2.14 x 10^{-2} M)	Prepared by dissolving 500mg of MBTH in 100mL distilled water.
HCL solution (1.0M)	Prepared by dissolving 8.6 mL of conc. HCl to 100mL distilled water and standardized.
NaOH solution (BDH, 0.4%, 0.1M)	Prepared by dissolving 400mg of NaOH to 100mL distilled water and standardized.
Fe (III) solution (0.25%, 1.53 x 10^{-2} M)	Prepared by dissolving 250mg of ferric chloride in 100mL distilled water.

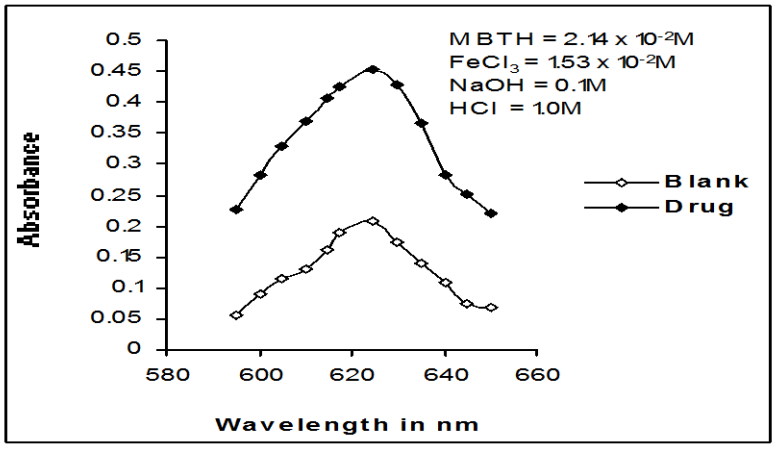


Fig. 2: Absorption spec of ATXT with MBTH - Fe(III) (M_{11})

Method M_3:

Brucine Solution (Loba; 0.2%, 5.067 x 10^{-3}M)	200mg of brucine was first dissolved in few drops of H_2SO_4 and then diluted to 100mL with distilled water.
$NaIO_4$ solution (BDH; 0.2%, 9.35 x 10^{-3}M)	Prepared by dissolving 200mg of sodium meta periodate in 100mL of distilled water and standardized iodometrically.
H_2SO_4 solution (Qualigens)	Prepared by adding 6.38mL of 18M H_2SO_4 to 93.62mL of with distilled water with constant shaking and cooling

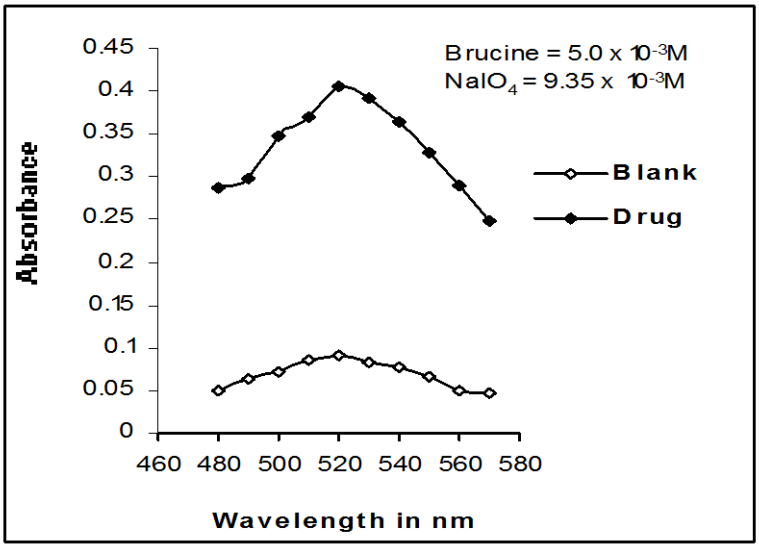


Fig 3: Absorption spec of ATXT with Brucine (M_{12})

Method M_4:

Potassium ferricyanide solution (BDH; 0.1%, $3.02x10^{-3}$ M)	Prepared by dissolving 100mg of $K_3Fe(CN)_6$ in 100mL of distilled water.
Fe(III) solution (Wilson labs; 0.054%,$3.32x10^{-3}$ M)	Prepared by dissolving 54mg of anhydrous ferric chloride in 100 mL of distilled water.
Hydrochloric acid (1N)	Prepared by diluting 86mL of concentrated HCl to 1000mL with distilled water and standardized.

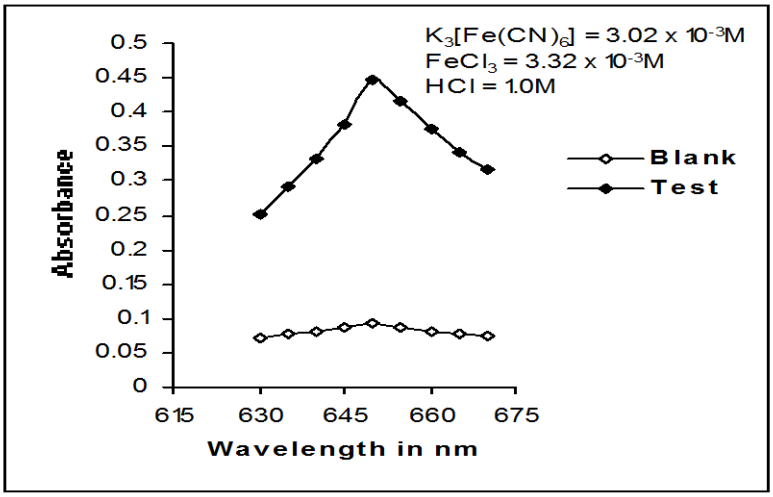


Fig 4: Absorption spec of ATXT with Fe(III) - $K_3Fe(CN)_6$ (M_{14})

Method M_5:

AV Solution (Loba; 5%, 2.618×10^{-2} M)	Prepared by dissolving 5gms of ammonium vanadate in 100mL of distilled water.
Conc.H_2SO_4 (Qualigens)	Used as it is.

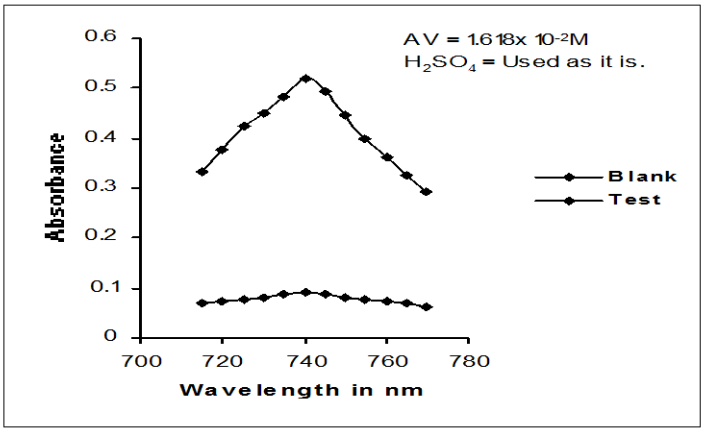


Fig. 5: Absorption spectrum of ATXT with AV (M_{15})

Proposed procedures: After systematic and detailed study of the various parameters involved, the following procedures **{Method M_1[MBTH – $NaIO_4$], M_2[MBTH - Fe(III)], M_3[Brucine-IO_4^-], M_4 [Fe(III) - $K_3Fe(CN)_6$], M_5[AV]}** were recommended for the assay of atomoxetine in bulk and pharmaceutical formulations.

Method – M_1 : To different aliquots of standard **ATXT** solution (0.5 – 3.0mL, 100µg.mL^{-1}), in a series of 25.0mL calibrated, 1.0mL of $NaIO_4$ and AcOH were added. The volume was made up to the mark with distilled water and the mixture was kept in boiling water bath for 40min. The solutions were cooled. After that 1mL of MBTH solution was added and kept aside for 15 min. After cooling the volume was made up to 25.0mL with distilled water. The absorbance was measured at 585nm against reagent blank. The amount of **ATXT** was computed from its calibration graph (**Fig.6**).

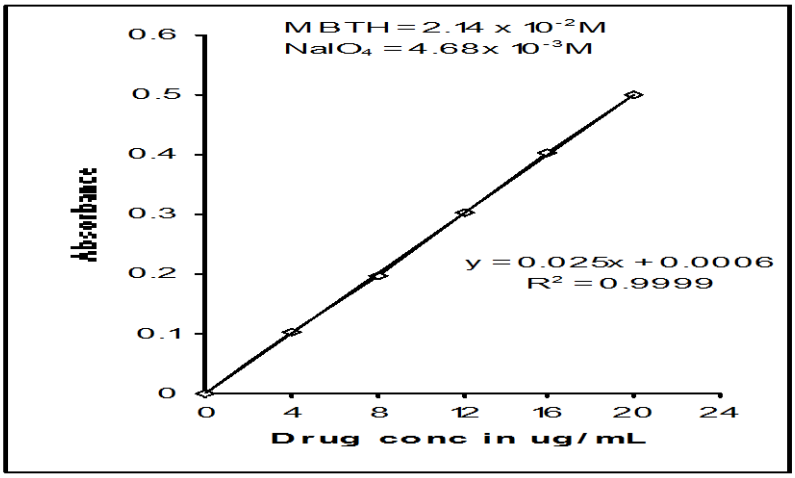


Fig. 6: Beer's Law plot of ATXT with MBTH-- $NaIO_4$ (M_{10})

Method – M_2 : Into a series of 10.0mL calibrated tubes aliquots of standard **ATXT** solution (0.5 – 2.5mL, 100µg/mL) wre transferred. To each tube 0.5mL of 2.14x10^{-2}M of MBTH solution, 0.5mL of 0.1M NaOH were added and the contents were heated for 10min. in a water bath at 100^{o}C and cooled for 5min. in a water bath at 15^{o}C, then 0.5mL of 1.0M HCl and 2.0mL of 1.53 x 10^{-2}M of Fe (III) solution were added successively and kept aside for 1hr. Finally the solution in each tube was made up to 10.0mL with distilled water. The absorbance was measured at 625nm against a similar reagent blank. The amount of **ATXT** was computed from its calibration graph. **(Fig.7)**.

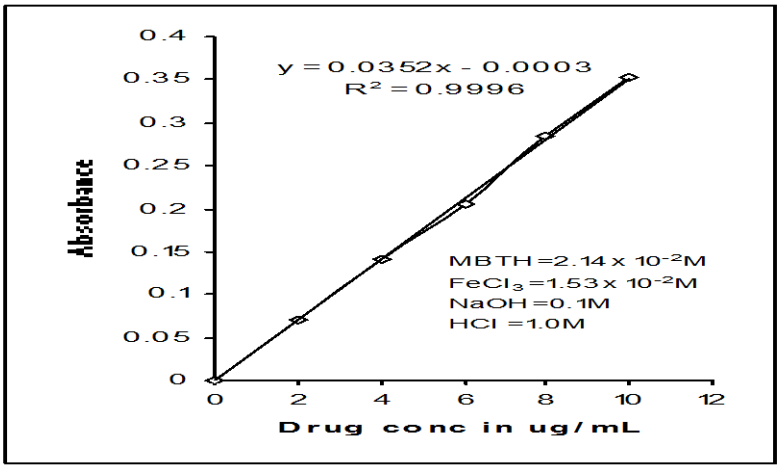


Fig. 7: Beer's Law plot of ATXT with MBTH-Fe(III) (M_{11})

Method – M_3 : Aliquots of **ATXT** solution (0.5 - 3.0mL, 500µg.mL^{-1}) were transferred into different 10.0mL graduated tubes 3.0mL (5.067 x 10^{-3}M) of brucine solution, 1.5mL (9.35x10^{-3}M) of sodium metaperiodate solution and 2.0mL (2.3M) of sulphuric acid were added to each tube and the total volume

was made up to 9.0mL with distilled water. The tubes were thoroughly shaken and placed in a boiling water bath for 15min. The reaction mixture was then cooled to room temperature and total volume was adjusted to 10.0mL with distilled water. The absorbance of each solution was measured at 520nm against a reagent blank. The amount of **ATXT** present in the sample was computed from the calibration graph (**Fig.8**).

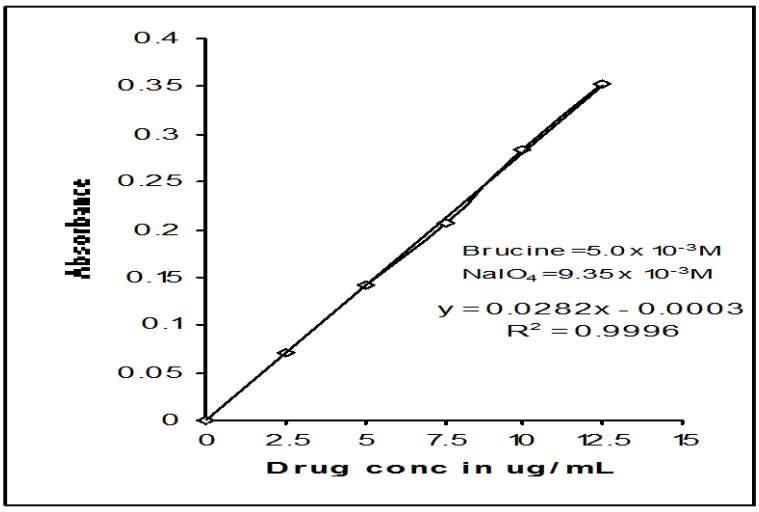


Fig.8: Beer's Law plot of ATXT with Brucine – $NaIO_4$ (M_{12})

Method – M_4 : Into a series of 10.0mL calibrated tubes ,aliquots of standard **ATXT** solution (0.5- 2.5 mL, 80µg.mL^{-1}) were transferred and 1.0mL of 3.32 x 10 $^{-3}$ M $FeCl_3$ solution was added .The tubes were stoppered immediately and shaken well for 5min. Then 0.5mL of 3.02x10^{-3} M potassium ferricyanide solution was added into each tube and was closed with lids immediately. After 5min. 1.0mL of 1 N HCl was added and the final volume was added upto 10.0mL with distilled water. The absorbance of the solution in each tube was measured immediately at 650nm against a similar reagent blank. The amount of the drug was calculated from its calibration graph (**Fig. 9**).

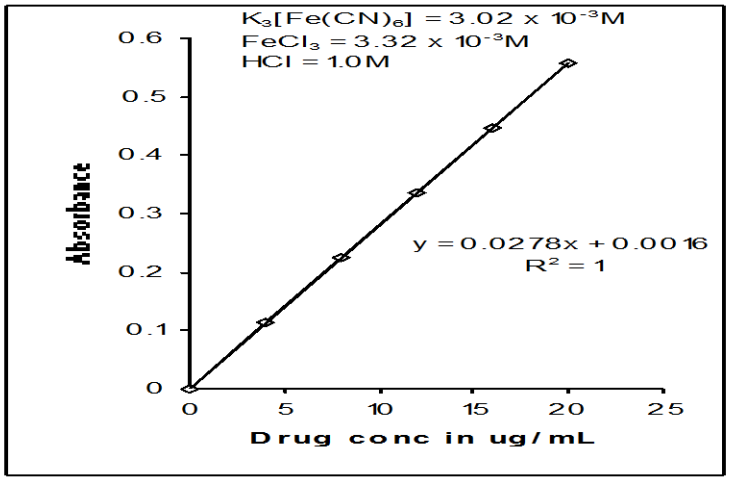


Fig. 9: Beer's Law plot of ATXT with Fe(III) - $K_3Fe(CN)_6$ (M_{14})

Method – M_5 : Aliquots of standard **ATXT** solution (0.5-2.5mL, 250µg.mL^{-1}) were delivered in to a series of 25mL calibrated tubes. To each tube 1.0mL of 1.618 x 10^{-2}M AV reagent and 4.0mL of

Conc.H_2SO_4 were added to each tube and the contents were heated for 20min. in boiling water bath. After cooling, the volume was made up to 25mL with ethanol. The resulting absorbance was measured at 740nm against a reagent blank. The amount of **ATXT** was computed from to appropriate calibration graph **(Fig.10)**.

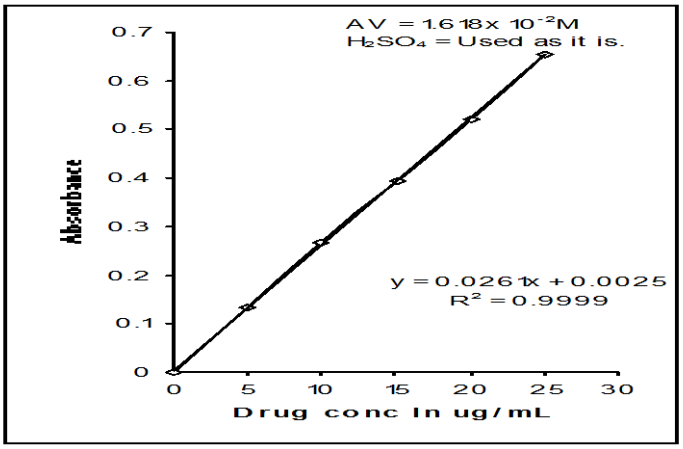


Fig. 10: Beer's Law plot of ATXTwith AV (M_{15})

RESULTS AND DISCUSSION

Spectral Characteristics: In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in the above methods, specified amounts of **ATXT** were taken and colors were developed separately by following the above procedures. The reagent blank absorption spectrum of each method was also recorded against distilled water.

Parameters fixation: The optimum conditions for the color development of methods (**M_1**, **M_2**, **M_3**, **M_{44}** and **M_5**) were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species and are tabulated in **tables.1 –5.**

Method – M_1 [MBTH – $NaIO_4$]: In developing this method, a systematic study of the effects of various parameters were undertaken by varying one parameter at a time and keeping the others fixed, which is the standard practice in this type of work. The studies for this purpose include volume of $NaIO_4$, time and temperature required for oxidation (prior to the addition of MBTH), volume of MBTH, effect of volume of AcOH, time and temperature for color development (after the addition of MBTH),order of addition, solvent for final dilution and stability of colored species formed were studied. The optimum conditions are incorporated in **table. 1.**

Table 1: Optimum conditions established in method M_1 for ATXT

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	570–620 for ATXT	585 for ATXT	
Effect of volume of ($9.35x10^{-3}$M) $NaIO_4$ solution required	0.9 -1.3mL	1.0mL	Addition of <0.9mL and>1.3mL results in low absorbance values.
Time and temp required for oxidation prior to the addition of MBTH	35 - 45 min on boiling water bath	40min on boiling water bath	Heating time of 40min. on boiling water bath was required to produce maximum color. At <30min complete oxidation wasd not possible
Effect of volume of ($8.56x10^{-3}$M) MBTH	0.8 - 1.4mL	1.0mL	Additionof <0.8mL resulted in low absorbance especially at higher Beer's

required for color development			law limits. Increasing the volume beyond 1mL has no effect.
Solvent for final dilution.	Water	Water	Distilled water is sufficient for final dilution, other water miscible solvents like methanol, ethanol,acetone etc.did not improve the color development to any extent.
Stability period after final dilution.	Immediately-45min	Immediate	

Method – M_2 [MBTH – Fe(III)] : The method involves the reaction of **ATXT** with MBTH in the presence of an oxidant (Fe (III) solution). The optimum conditions in this method were fixed, basing on the study of the effects of various parameters such as MBTH and alkali to be added to accompanied by heating (time and temperature), acid and oxidant required after cooling, the waiting time for maximum color development and solvent used for final dilution on the intensity and stability of the colored species formed. The optimum conditions developed and actual conditions chosen for the procedure are incorporated in **table 2.**

Table 2: Optimum conditions established in method M_2 for ATXT

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	610 - 650	625	
Nature of oxidant for color development	Fe(III) solution	Fe(III) solution	Other oxidants such as Ce (IV), Cr (VI), CAT, IO_4^- and $S_2O_8^{2-}$ were used instead Fe (III), resulting in the decrease in absorbance were noticed.
Volume of ($2.14x10^{-2}$M) of MBTH on color development.	0.2 - 0.8mL	0.5mL	<0.2mL decreased the absorbance of the test solution. The absorbance of the colored species remained stable with rise in volume of MBTH up to 0.8mL.
Effect of volume of (0.1M) NaOH solution.	0.2 – 1.0mL	0.5mL	Minimum amount of 0.2mL of NaOH solution was necessary to maintain alkaline conditions.
Temperature and time.	Boiling water bath, 5 - 20min.	Boiling water bath,15min.	Heating on a boiling water bath for 10min. has been preferred.
Effect of volume of (1.0M) HCl solution.	0.2 - 1.0mL	0.5mL	Minimum amount of 0.2mL of HCl solution was necessary to maintain acidic conditions.
Volume of (1.53×10^{-2}M) Fe(III) solution required for color development.	1.5 - 2.5mL	2.0mL	<1.5mL of Fe(III) solution decreased the absorbance of test solution and >2.5mL of Fe (III) solution increased the blank absorbance.
Stability period after final dilution.	Immediate-60min.	5min.	After the stability period, the intensity of the colored species was found to decrease with time after 60min.

Method – M_3 [MBTH – Fe(III)] : The method involves the reaction of **ATXT** with MBTH in the presence of an oxidant (Fe (III) solution). The optimum conditions in this method were fixed, basing on the study of the effects of various parameters such as MBTH and alkali to be added to accompanied by heating (time and temperature), acid and oxidant required after cooling, the waiting time for maximum color development and solvent used for final dilution on the intensity and stability of the colored species formed. The optimum conditions developed and actual conditions chosen for the procedure are incorporated in **table.2.**

Method – M_4 [Brucine – $NaIO_4$] : The method involves the reaction of **ATXT** with brucine in the presence of sodium metaperiodate. The effect of various parameters such as the volume of brucine, the

volume of oxidant, strength of the acid, the temperature, and solvent for final dilution and stability of the colored species were studied. The optimum conditions are incorporated in **table. 3.**

Table 3: Optimum conditions established in method M_3 for ATXT

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	500 - 530	520	
Effect of volume of ($5.067x10^{-3}$M) brucine on colored development.	2.5 - 3.5mL	2.0mL	Addition of brucine at lower limits results in low absorbance. In creasing the volume beyond upper limits has no effect.
Effect of volume ($9.35x10^{-3}$M) of $NaIO_4$ required for maximum color development.	1.2 - 1.8mL	1.5mL	<1.2mL of $NaIO_4$ results in decrease of absorbance and > 1.8 mL results in development of intense rose color in the blank.
Effect of volume of sulphuric acid (2.3M) on color development.	1.8 - 2.3mL	2.0mL	2.0mL of 2.3 M sulphuric acid was necessary for attaining maximum color and stability.
Nature of oxidant on color development.	$NaIO_4$	$NaIO_4$	Other oxidants such as Fe(III), Cr(VI), V(V), IO_3^- and $S_2O_8^{2-}$ were tried in the place of IO_4^-but were found to be inferior.
Effect of temperature on colored species.	Boiling water bath	Boiling water bath	It was found that boiling water bath was necessary for uniform temperature and maximum color development. Below this temperature the intensity of the colored species was less.
Effect of heating time.	10 - 20min	15min	Below 10min, the intensity of the colored species was less.
Stability period after dilution.	1 - 45min	10min	After the stability period, the intensity of the colored species was found to decrease with time.

Method – M_5 [Fe(III) – $K_3Fe(CN)_6$] : The optimum conditions in this method were fixed basing on the study of the effects of various parameters such as volumes of $3.32x10^{-3}$ M ferric chloride solution, $3.02x10^{-3}$ M potassium ferricyanide solution and 1N HCl, time and temperature necessary for complete color development, the stability and intensity of the colored species after final dilution were established by measuring absorbance's at 700nm and results were incorporated in **table 4.**

Table.4: Optimum conditions established in method M_4 for ATXT

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	680 - 730	650	-
Volume of ferric chloride ($3.32x10^{-3}$M) required for oxidation.	0.8 - 1.5mL	1.0mL	Optimum conditions furnished in column 2 were preferred for broad coverage of Beer's Law limits and stability of colored species formed.
Vol.ofPotassiumferricyanide ($3.02x10^{-3}$M) for formation of ferrous ferricyanide.	0.4 - 0.7mL	0.5mL	
HCl (1N) necessary for maintenance of acidity prior to dilution.	0.8 - 1.5mL	1.0mL	-
Temperature and time necessary for complete development of color.	5 - 15min. Room temp.	10min. Room temp.	-
Stability period	Immediate -1hr.	Immediate-1hr.	-

Method – M_{15} [AV]: The method involves the reaction of the drug **ATXT** with AV in acid medium. The effect of various parameters, such as conc and volume of AV, nature and strength of acid, order of addition of reagents, solvent for final dilution were studied and the optimum conditions developed and recorded in **table.5**.

Table 5: Optimum conditions established in method M_5 for ATXT

Parameter	Optimum range	Conditions in procedure
λ_{max} (nm)	740 – 780	760
Effect of volume of $2.61x10^{-2}$M of AV solution.	0.7 - 1.3mL	1.0mL
Effect of volume of Conc. H_2SO_4 on color development	3.0-5.0mL	4.0mL
Effect of the order of addition of reagent on color development	ATXT,AV solution, Conc. H_2SO_4	ATXT,AV solution, Conc. H_2SO_4
Effect of temperature and time	Boiling water bath 20-30min.	Boiling water bath 20min.
Solvent for final dilution	Ethanol	Ethanol
Stability period after final dilution	5min-24 hours	5min.

Optical Characteristics: In order to test whether the colored species formed in the above methods, adhere to Beer's law, the absorbances at appropriate wavelengths of a set of solutions containing varying amounts of **ATXT** and specified amounts of reagents (as given in the recommended procedures for each method) were recorded against the corresponding reagent blanks. The Beer's law plots of these systems are recorded against the corresponding reagent blanks. The Beer's law plots of these systems are recorded graphically **(Figs. 6 to 10,)**. Beer's law limits, molar absorptivity, sandell's sensitivity and optimum photometric range for **ATXT** with each of the mentioned reagents were calculated and are recorded **(Table. 6)**. Least square regression analysis was carried out for getting the slope, intercept and correlation coefficient and the values are recorded in **(Table 6)**.

Precision: The precision of each proposed method was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of **ATXT** in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods are incorporated in **(Table. 6)**.

Accuracy: To determine the accuracy of each proposed method, different amounts of bulk samples of **ATXT** within the Beer's law limits were taken and analyzed by the proposed methods. The results are recorded in **(Table. 6)**.

Table.6: Optical and regression characteristics, precision and accuracy of the proposed methods for Atomoxetine

Parameter	M_1	M_2	M_3	M_4	M_5
λ_{max} (nm)	585	625	520	650	740
Beer's law limits (µg/mL)	4.0 – 20.0	2.0 – 10.0	2.5 – 12.5	5.0 – 25.0	5.0 – 25.0
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	5.132×10^5	3.600×10^5	1.260×10^4	5.694×10^5	5.327×10^5
Sandell's sensitivity (µg.cm$^-$	0.0497	0.0709	0.0202	0.0448	0.0479

^{2}/0.001 absorbance unit)					
Optimum photometric range (µg/mL)	5.0 – 15.0	3.5 – 7.5	3.0 - 12.0	5.0 – 20.0	5.0 – 20.0
Regression equation (Y=a+bc) ;Slope (b)	0.025	0.0352	0.0243	0.0271	0.0261
Intercept (a)	0.0006	0.0003	0.0035	0.0016	0.0025
Correlation coefficient (r)	0.9999	0.9996	0.9994	1.0000	0.9999
Relative standard deviation (%)	0.4825	1.3566	0.7886	0.4365	0.3712
% Range of error (confidence limits)					
0.05 level	0.4035	1.1345	0.6594	0.3650	0.3105
0.01 level	0.5936	1.6781	0.9755	0.5399	0.4596

*Average of six determinations considered

Interference studies: The effect of wide range of excipients and other active ingredients usually present in the formulations for the assay of **ATXT** in methods **(M_1, M_2, M_3, M_4** and **M_5)** under optimum conditions were investigated. The commonly used excipients and other active ingredients usually present in formulations did not interfere even if they were present in amount than they usually exist.

Nature of the colored species: The functional groups such as aliphatic secondary amine **(M_1, M_2, M_3, M_4** and **M_5)** present in Atomoxetine (ATXT) were exploited for developing the proposed methods. The nature of color species formed in each one has been explained basing on the analogy and probability.

Method - M_{10} : In this reaction, Atomoxetine gives oxidative coupled product with MBTH in the presence of an oxidant [IO_4^-]. Under the reaction condition MBTH looses two electrons and one proton during oxidation to form an electrophilic intermediate, which is the active coupling species. These active species reacts with the coupler (i.e.) by electrophilic attack on the most nucleophilic site of the coupler. The probable sequence of the reactions, based on analogy is presented in **Scheme-1.**

Method - M_{11} : In this reaction, Atomoxetine gives oxidative coupled product with MBTH in the presence of an oxidant, Fe(III). Under the reaction condition MBTH looses two electrons and one proton during oxidation to form an electrophilic intermediate, which is the active coupling species. These active species reacts with the coupler (i.e.) by electrophilic attack on the most nucleophilic site of the coupler. The probable sequence of the reactions, based on analogy is presented in **Scheme-2.**

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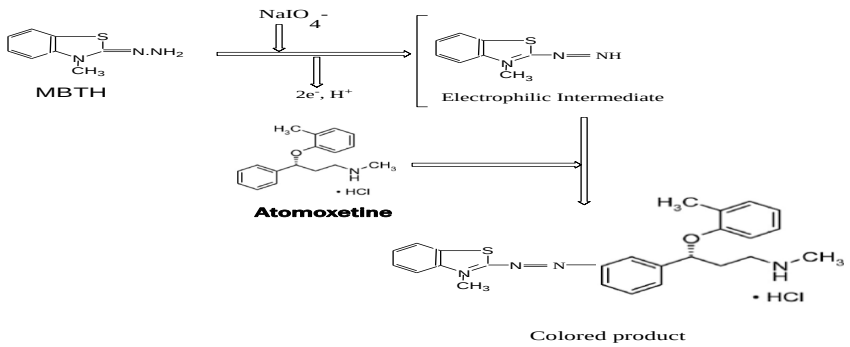
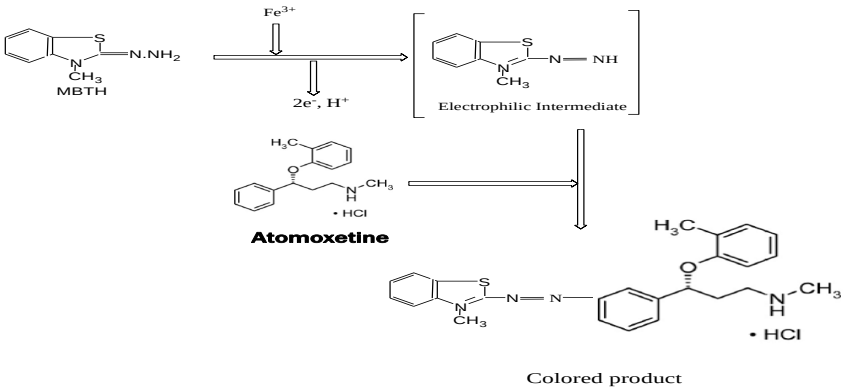
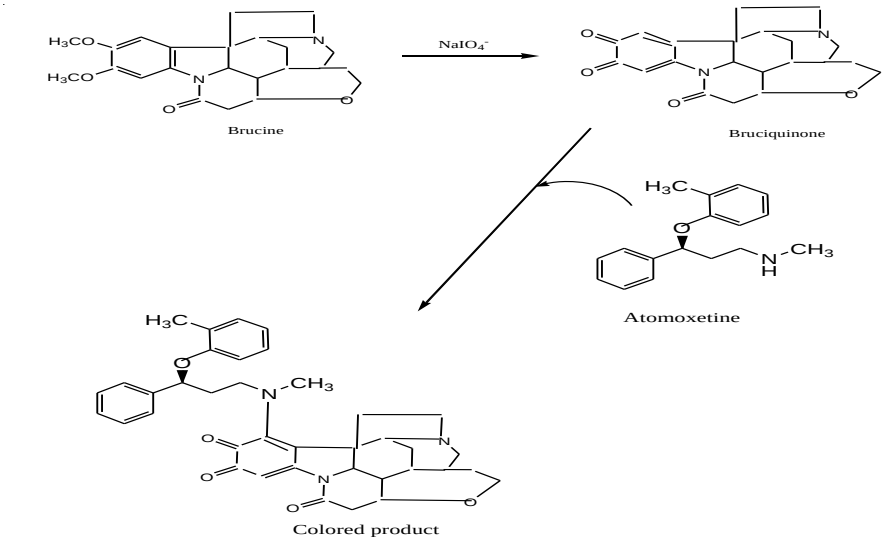


Scheme- 1



Scheme -2

Method- M_{12}: The dimethoxy benzene nucleus of brucine is attacked by IO_4^- with the formation of o-quinine (bruciquinone), which in turn undergoes nucleophilic attack on the most electron rich portion of the coupler (secondary nitrogen) to give 1-monosubstituted bruciquinone derivative. The reaction of ATXT with brucine in the presence of IO_4^- is described in **Scheme -3**.



Scheme-3

Method –M_{14}: This method is based on the oxidation of Atomoxetine by excess ferric salt to give products of oxidation inclusive of Fe (II) (reduced form of oxidant).The reduced form of oxidant which subsequently reacts with ferricyanide to give ferrous ferricyanide.

Step I:

Atomoxetine + Fe (III) → oxidation products + Fe (II) + unreacted Fe (III)

Step II: the second step concerns with the estimation of Fe (II) with $[Fe(CN)_6]^{3-}$.

$$3Fe^{2+} + 2[Fe(CN)_6]^{3-} \rightarrow Fe_3[Fe(CN)_6]_2$$

Method - M_{15} : Reducible groups present in the A**tomoxetine** probably effects the reduction of 1,2 or 3 oxygen atoms from exemplified vanadate, thereby producing one or two more of possible reducing species which have a characteristic intense blue color.

APPLICATIONS

Procedure for the assay of Atomoxetine in Pure and Pharmaceutical dosage forms:
An accurately weighed portion of powdered tablets equivalent to 100mg of **ATXT** was dissolved in 20mL of methanol, shaken well and filtered, the filtrate was diluted to 100mL with MeOH to get 1mg mL^{-1} of drug in formulations.5.0mL of this solution was diluted to 100mL to get 50µg mL L^{-1}. The absorbance of the solution was determined at λ_{max} 220nm.The quantity of was computed from Beers law of standard drug in MeOH and were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically with F and t-tests. The results of the recovery experiments by the proposed methods are also listed in **(Table 7).**

Table 7: Assay of Atomoxetine in Pharmaceutical Formulations

Formulations*	Amount taken (mg)	Amount found by proposed Methods**					Reference method	Percentage recovery by proposed methods***				
		M_1	M_2	M_3	M_4	M_5		M1	M_2	M_3	M_4	M_5
Tablet I (Stratteera)	10	9.92±0.04 F=4.00 t=1.44	9.90±0.07 F=1.306 t=1.616	9.91±0.06 F=1.777 t=1.484	9.93±0.05 F=2.56 t=1.06	9.88±0.07 F=1.306 t=2.078	9.97±0.08	99.59±0.41	99.09±0.22	99.39±0.36	100.50±0.68	99.09±0.65
Tablet II (Stratteera)	18	17.84±0.18 F=1.361 t=0.977	17.87±0.11 F=3.644 t=0.866	17.83±0.16 F=1.722 t=1.123	17.81±0.14 F=2.25 t=1.38	17.85±0.11 F=3.644 t=1.082	17.95±0.21	99.44±0.86	99.59±0.55	99.39±0.16	99.29±0.47	99.49±0.82

* Tablets from four different pharmaceutical companies, ** Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method, Theoretical values at 95% confidence limit, F = 5.05, t = 2.262, *** Recovery of 10mg added to the preanalysed pharmaceutical formulations (average of three determinations).

CONCLUSIONS

The described spectrophotometric method was applied in the assay of Atomoxetine in bulk and pharmaceutical formulations. The method is simple, accurate and reproducible. The statistical analysis has good agreement with reported methods. The optimum conditions for the proposed method have been established and the method has shown a reasonable tolerance towards excipients. Finally, the proposed method can be employed for the routine analysis of Atomoxetine from bulk and tablet dosage form in quality control laboratories due to the minimum time required for the complexation to be complete.

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