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SIMPLE AND CONVENIENT VISIBLE SPECTROPHOTOMETRIC ASSAY OF ATOMOXETINE HYDROCHLORIDE IN BULK DRUG AND PHARMACEUTICAL PREPARATIONS

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ABSTRACT

Two direct, simple and sensitive visible spectrophotometric methods (M_1 & M_2) are described for the assay of atomoxetine hydrochloride in pure and solid dosage forms. The method M_1 involves oxidative coupling of atomoxetine with brucine in presence of sodium meta periodate and purple red colored species is formed and exhibits absorption maxima at 520.5 nm. The method M_2 is based on the formation of yellowish brown colored species by the drug with Folin reagent and exhibits absorption maxima at 450.6 nm. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges (4.0-20) µg/mL for method M_1, (16-48) µg/mL for method M_2 respectively. The proposed methods are applied to commercial available tablets and the results are statistically compared with those obtained by the UV reference method and validated by recovery studies. The results are found satisfactory and reproducible. These methods are applied successfully for the estimation of the atomoxetine hydrochloride in the presence of other ingredients that are usually present in dosage forms. These methods offer the advantages of rapidity, simplicity and sensitivity and normal cost and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.

Key words: ADHD, BCN-IO_4^-, Folin reagent, Nucleophillic substitution, Oxidative coupling, Statistical analysis.

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INTRODUCTION

The atomoxetine hydrochloride (ATH) (Fig. 1) is the first non-stimulant drug approved by United States FDA for symptomatic treatment of attention-deficit hyperactivity disorder (ADHD) and selective noradrenaline (norepinephrine) reuptake inhibitor (NRI). It is chemically known as (*R*)-*N*-methyl-3-(*o*-tolyloxy)-3-phenylpropyl amine hydrochloride[1-2]. The drug is used in the treatment of depression. Its empirical formula is $C_{17}H_{21}NO.HCl$ and its molecular weight is 291.82. The drug is not yet official in any Pharmacopoeia. ATH is a norepinephrine transport inhibitor that acts almost exclusively on the noradrenergic pathway. Its mechanism of action in the control and maintains of ADHD symptoms is thought to be through the highly specific presynaptic inhibition of norepinephrine.

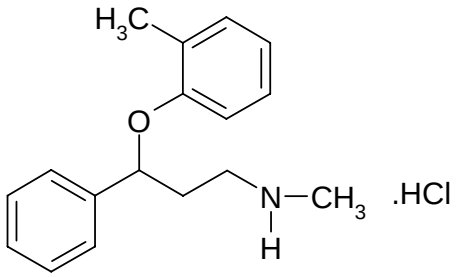


Fig. 1: Chemical structure of ATH

In the literature, several analytical techniques like GC[3], HPLC[4-12], HPTLC[13], LC-MS-MS[14], chemiluminescence[15], X-ray powder diffraction[16], voltammetry[17] and UV spectrophotometry[18-20] have been reported for its determination in plasma and capsule dosage forms. For routine analysis, simple, rapid and cost effective visible spectrophotometric methods are required and preferred. As on date no visible spectrophotometric methods have been reported for estimation of ATH in bulk drug and formulations. So the authors have made some attempts in developing visible spectrophotometric methods and succeeded in developing two methods based on the reaction between the drug and BCN-IO_4^- reagent[21] (M_1) or folin reagent[22] (M_2) under specified experimental conditions.

The proposed methods for ATH determination have many advantages over other analytical methods due to its rapidity, normal cost and environmental safety. Unlike HPLC, HPTLC procedures, the instrument is simple and is not costly. Economically, all the analytical reagents are inexpensive and available in any analytical laboratory. These methods can be extended for the routine quality control analysis of pharmaceutical products containing ATH.

EXPERIMENTAL

Materials and methods

Apparatus and chemicals

A Shimadzu UV-Visible spectrophotometer 1601 with 10 mm matched quartz cells was used for all spectral measurements. A Systronics digital pH meter Model-361 was used for pH measurements. All the chemicals used were of analytical grade. Pure ATH drug was obtained as a gift sample from M/s Tychy Industries, Hyderabad (AP). Axepta-25 mg tablets and Attentrol-10 mg capsules were purchased from local market.

Aqueous solution of Brucine (Loba, 0.2%, 5.067 x 10^{-3} M prepared by dissolving 200 mg of brucine initially in minimum amount of 0.16 M sulphuric acid and then made upto 100 mL with distilled water), sodium metaperiodate (BDH, 0.2%, 9.35 x 10^{-3} M prepared by dissolving 200 mg of sodium metaperiodate in 100 mL distilled water and standardized iodometrically) and sulphuric acid (Qualigens, 1.2 M prepared by diluting 126 mL of conc. H_2SO_4 to 100 mL of distilled water initially, followed by diluting to 1000 mL with distilled water) were prepared for method M_1.

Folin reagent (NQS) solution (Loba, 0.5%, 1.92 x 10^{-2} M prepared by dissolving 500 mg of NQS in 100 mL of distilled water), pH 8.0 buffer solution (prepared by mixing 30 mL of potassium hydrogen phosphate (0.067 M) and 970 mL of disodium hydrogen phosphate (0.067 M) and the pH of the solution was adjusted to 8.0) were prepared for method M_2.

Preparation of standard stock solution

The standard stock solution (1 mg/mL) of ATH was prepared by dissolving 100 mg of ATH in 100 mL distilled water. The working standard solutions of ATH were obtained by appropriately diluting the standard stock solution with the same solvent (M_1- 200 µg/mL & M_2- 400 µg/mL). The prepared stock solution was stored at 4°C protected from light. From this stock solution, a series of standards were freshly prepared during the analysis day.

Preparation of sample solution

About 20 tablets or capsules were weighed to get the average tablet or capsule weight and pulverized. The powder equivalent to 100 mg of ATH was weighed, dispersed in 25 mL of isopropyl alcohol, sonicated for 15 minutes and filtered through Whatman filter paper No. 41. The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparation.

Determination of wavelength maximum (λ_{max})

Method M_1: The 2.5 mL of working standard solution of ATH (200 µg/mL) was taken in 25 mL calibrated tube. To this, 3.0 mL brucine, 1.5 mL of $NaIO_4$ solution and 2.0 mL of sulphuric acid were added successively and the volume was brought up to 10 mL with distilled water and kept in boiling water bath for 15 min. for complete color development. The solution was cooled to room temperature and the volume was made up to the mark with distilled water. In order to investigate the wavelength maximum, the above colored solution was scanned in the range of 400-760 nm UV-Visible spectrophotometers against a reagent blank. From the absorption spectra (Fig. 2), it was concluded that 520.5 nm is the most appropriate wavelength for analyzing ATH with suitable sensitivity.

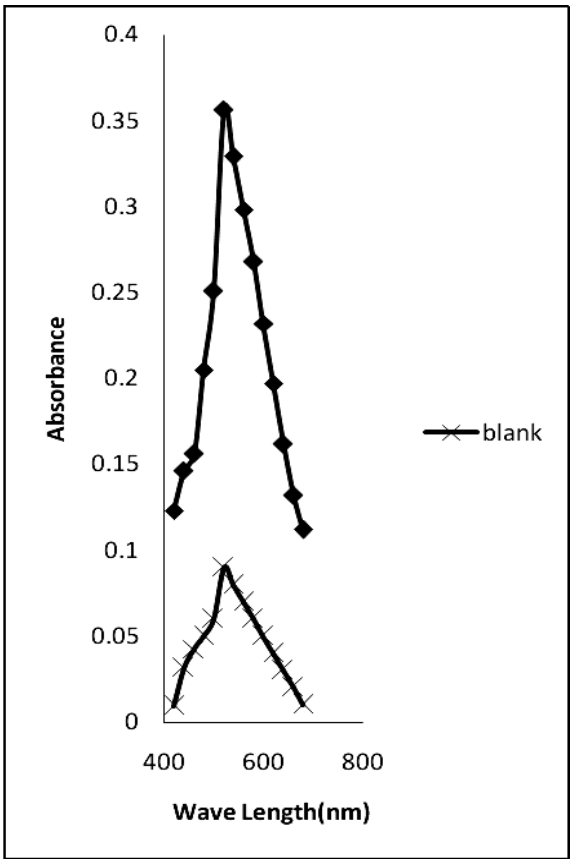


Fig. 2: Absorption spectra of ATH-BCN-IO_4^-

Method M_2: The 3.0 mL of working standard solution of ATH (400 µg/mL) was taken in 25 mL standard flask. To this, 1.0 mL of folin reagent (1.092×10^{-2} M), 5.0 mL of

pH 8.0 buffer and 1.5 mL of distilled water were added and kept aside for 15 min for complete color development. Then the volume was made up to 25 mL using distilled water and sonicated for 1 min. In order to investigate the wavelength maximum, the above standard stock solution was scanned in the range of 360-560 nm by UV-Visible spectrophotometer. From the spectra (Fig. 3), it was concluded that 450.6 nm is the most appropriate wavelength for analyzing ATH with suitable sensitivity.

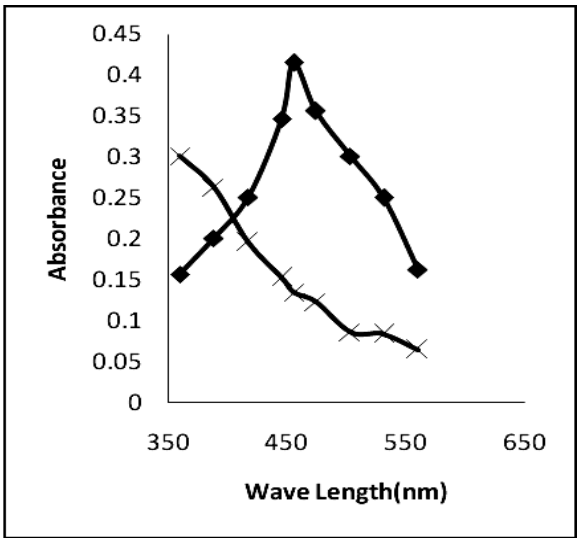


Fig. 3: Absorption spectra of ATH-NQS

Preparation of calibration curve

Aliquots of the standard ATH solution [0.5-2.5 mL, 200 µg/mL (method M_1) and 1.0-3.0 mL, 400 µg/mL (method M_2)] were placed in a series of 25 mL standard flask. Then 3.0 mL brucine, 1.5 mL of $NaIO_4$ solution and 2.0 mL of sulphuric acid were added successively and the volume was brought up to 10 mL with distilled water and kept in boiling water bath for 15 min. for complete color development. The solution was cooled to room temperature and the volume was made up to the mark with distilled water (method M_1) or 1.0 mL of folin reagent (1.092×10^{-2} M), 5.0 mL of pH 8.0 buffer and 1.5 mL of distilled water were added and kept aside for 15 min for complete color development. Then the volume was made up to 25 mL using distilled water and sonicated for 1 min. (method M_2) The absorbance was measured at 520.5 nm (method M_1) or 450.6 nm (method M_2) against a reagent blank within the stability period (5 minutes to 30 min). The calibration graph was constructed by plotting the drug concentration versus absorbance (Fig. 4 and 5). The amount of drug was computed from its calibration graph.

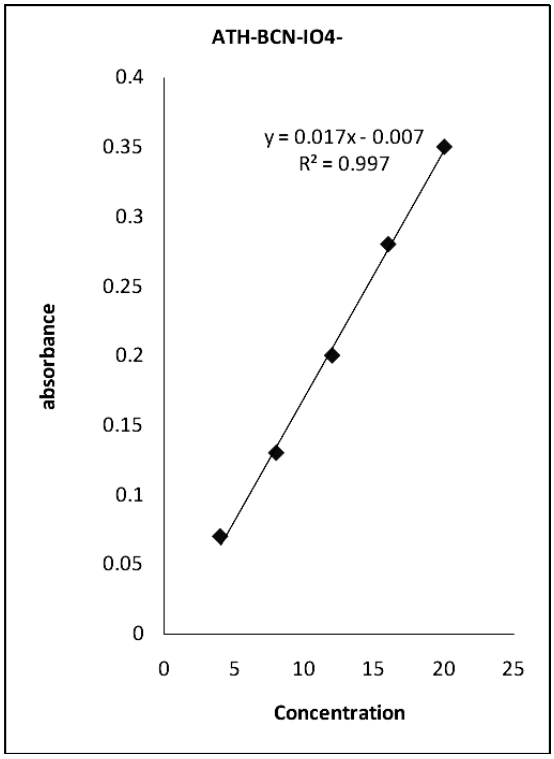


Fig. 4: Beer's Law Plot of ATH - BCN-IO_4^-

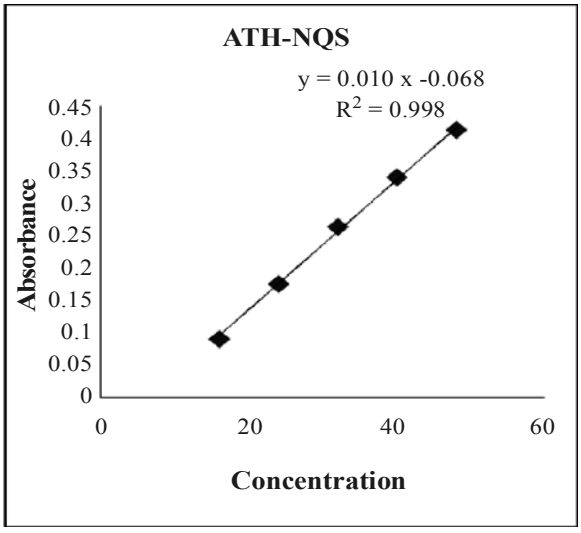


Fig. 5: Beer's Law plot of ATH-NQS

RESULTS AND DISCUSSION

In the present investigation, the presence of aliphatic secondary amino group of ATH permits the development of visible spectrophotometric methods for its determination through the oxidative coupling reaction with BCN-IO_4^- reagent (M_1) or the nucleophillic substitution with folin reagent (M_2).

Optimum operating conditions used in the procedure were established by adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time, volume and strength of reagents, the order of addition of reagents, pH buffer solutions and solvent for final dilution of the colored species were studied. The other oxidants such as Fe (III), Cr (IV), IO_3^-, and $S_2O_8^{-2}$ were tried in place of $NaIO_4$ and found to be inferior incase of method M_1. Distilled water was found to be best solvent for final dilution. Other water miscible solvents like methanol, ethanol, propan-2-ol and acetonitrile have no additional advantage in increasing the intensity of the color in both methods. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements containing $3/4^{th}$ of the amount of the upper Beer's law limits), Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and the results are summarized in Table 1.

Table 1: Optical characteristics, precision and accuracy of proposed methods

Parameter	Method M_1	Method M_2
λ_{max} (nm)	520.5	450.6
Beer's law limit (µg/mL)	8-24	16-80
Sandell's sensitivity (µg/cm^2/0.001 abs. unit	0.0024	0.00486692
Molar absorptivity (Litre/mole/cm)	121591.6667	59959.89663
Correlation coefficient regression equation (Y)*	0.997	0.998
Intercept (a)	-0.007	-0.068
Slope (b)	0.017	0.01
% RSD	1.149	1.576

Cont…

Parameter	Method M_1	Method M_2
% Range of errors (95% Confidence limits)		
0.05 significance level	1.2064	1.653
0.01 significance level	1.891	2.59

*Y = a + b x, where Y is the absorbance and x is the concentration of ATH in µg/mL

Commercial formulations containing ATH were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically by the t-and F-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. These results are summarized in Table 2.

Table 2: Analysis of Atomoxetine hydrochloride in pharmaceutical formulations by proposed and reference methods

Method	*Formulations	Labeled amount (mg)	Found by proposed methods			Found by reference method ± SD	#% Recovery by proposed method ± SD
			**Amount found ± SD	t	F		
M_1	Batch-1	25	24.96 ± 0.0279	0.408	4.086	24.97 ± 0.056	99.84 ± 0.1116
	Batch-2	10	9.95 ± 0.0539	0.585	2.20	9.98 ± 0.036	99.80 ± 0.216
M_2	Batch-1	25	24.969 ± 0.0456	0.232	1.527	24.97 ± 0.056	99.876 ± 0.1825
	Batch-2	10	9.979 ± 0.0305	0.0132	1.423	9.98 ± 0.036	99.92 ± 0.122

*Batches- 1 & 2 from two different companies (Batch-1: Axepta tablets from Intas pharmaceuticals; Batch 2: Attentrol capsules from Sun pharmaceuticals).

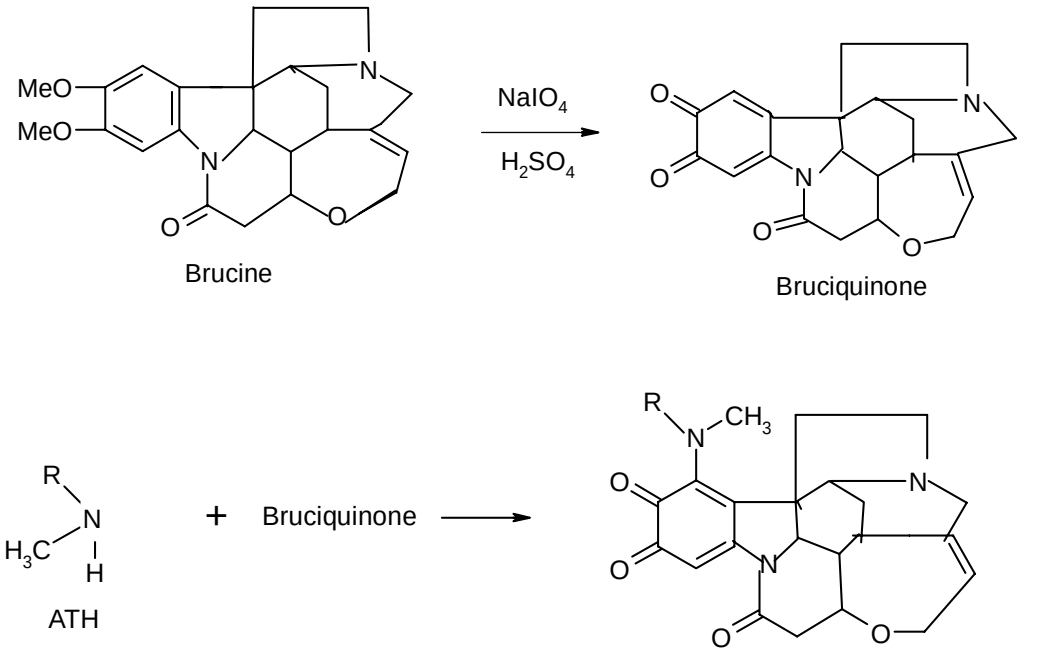
**Average ± Standard deviation of six determinations, the t- and f-values refer to comparison of the proposed method with reference method (UV). Theoretical values at 95% confidence limits t = 2.57 and f = 5.05.

#Recovery of 10 mg added to the pre-analyzed sample (average of three determinations).

Reference method (reported UV method) using double distilled water (λ_{max} = 270.5 nm).

Chemistry of colored species

In method M_1, the dimethoxy benzene nucleus of brucine is attacked by IO_4^- with the formation of o-quinone (bruciquinone) which in turn undergo nucleophillic attack on the most electron-rich position of the coupler (aliphatic secondary amino group of ATH) to give 1-monosubstituted bruciquinone derivative (purple red colored species). In method M_2, yellowish brown colored species (N-alkyl amino napthaquinone) was formed by replacement of the sulphonate group of the napthaquinone sulphonic acid by a secondary amino group of drug. The formation of colored species with these reagents may be assigned through above analogy as shown in Scheme (Fig. 6).



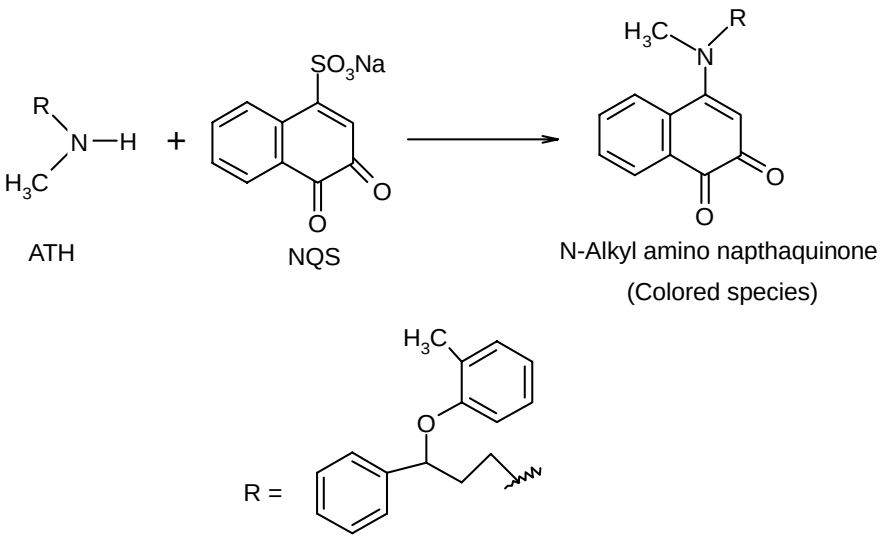


Fig. 6: Probable scheme of the reactions for methods M_1 &M_2

CONCLUSION

The proposed methods applicable for the assay of drug, the advantage of wider range under Beer's law limits, validated as per ICH guide lines and possess reasonable precision, accuracy, simple, sensitive. These methods can be extended for the routine assay of ATH formulations.

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