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Visible Spectrophotometric Methods for the Quantitative Estimation of Milnacipran in their Formulations

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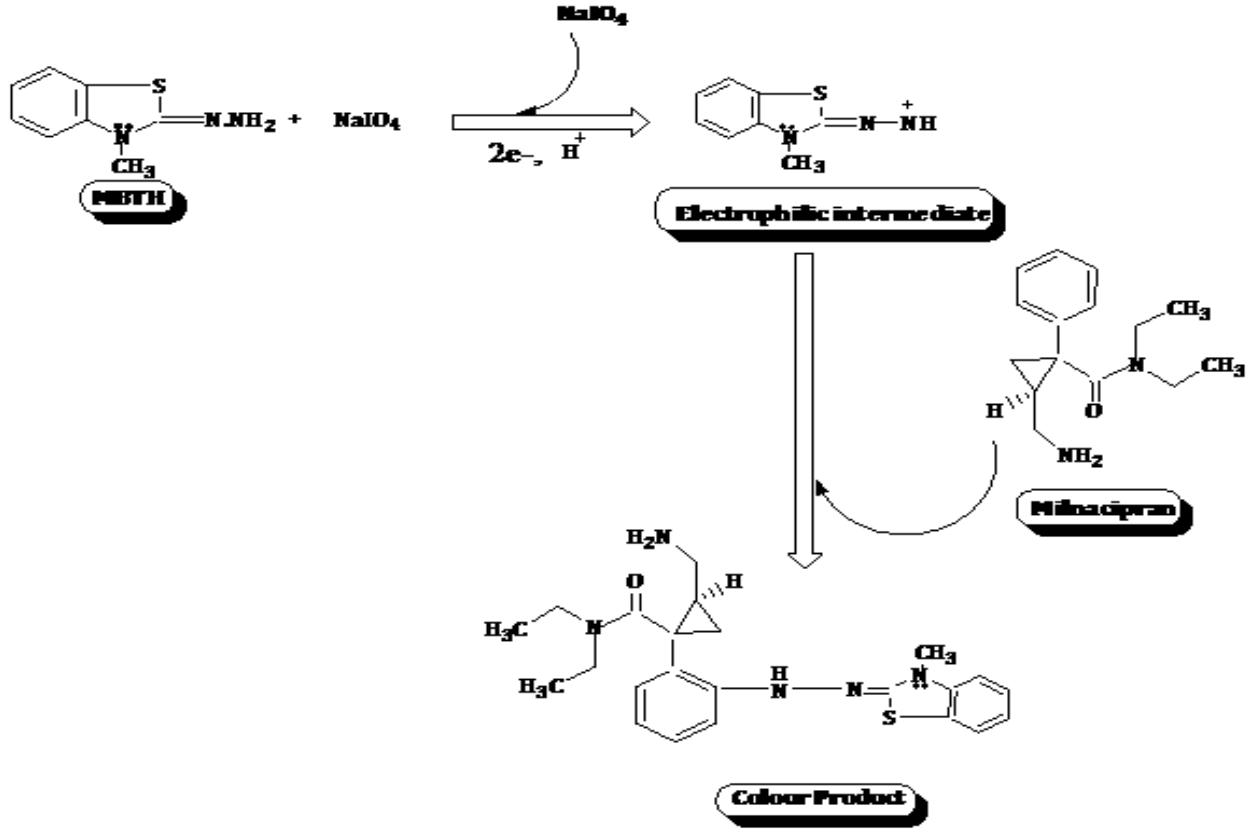
Abstract: Two simple, sensitive and economical visible spectrophotometric methods were developed for the analysis of Milnacipran in pure form as well as in pharmaceutical formulations. The Method-A is based on the formation of oxidation of 3-Methyl-2-benzothiazolinone hydrazone Hydrochloride (MBTH) with sodium meta periodate followed by coupling with mentioned drug forming a highly stable violet colored chromogen measured at 650nm The Method-B is based on redox reaction between ammonium vanadate (AV) and mentioned drug in presence of acidic medium to from a colored chromogen measured at 740 nm. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of proposed methods. The methods were successfully applied to the determination of Milnacipran in pharmaceutical formulations.
Key words: Milnacipran, MBTH, sodium meta periodate, ammonium vanadate and recovery studies.

INTRODUCTION

Milnacipran is chemically designated as (1R*,2S*)-2-(aminomethyl)-N,N-diethyl-1-phenylcyclopropane carboxamide. Milnacipran is the first in a new class of serotonin-norepinephrine reuptake inhibitor (SNRI)[1-3] A new medication Milnacipran has been shown to be very effective at treating chromic paid conditions, and currently being evaluated for treatment of the fibromyalgia syndrome was shown to prove. Some liquid chromatographic (LC) methods for determination of milnacipran combined with other antidepressants in human plasma have already been published [4-8]. A micellar electrokinetic capillary chromatographic method was developed for separation and determination of antidepressants and their metabolites in biological fluids[9] and LC enantio separation of milnacipran was investigated on different cellulose-based chiral stationary phases[10] and [RP-HPLC][5] existing analytical techniques reveal that little attention was paid in developing visible spectrophotometric methods by exploring thoroughly the useful functional groups in milnacipran. The present paper describes two visible spectrophotometric methods based on the reactivity of different functional groups present in the drug with the given reagents involving oxidation coupling and redox reactions. The developed two methods above are extended to pharmaceutical formulations as well.

SCHEME – I (Method –A)



SCHEME – II (Method –B)

The reduced form of Fe (III) (i.e. Fe (II) has a tendency to give a colored complex on treatment with $[Fe (CN)_6]^{3-}$.

<u>Step I</u>:

MCN + Fe(III) oxidation products + Fe (II) + unreacted Fe (III)

<u>Step II</u>:

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

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

EXPERIMENTAL

All spectral measurements were made on ELICO SL 159 Double beam UV-visible spectrophotometer. All chemicals used are of analytical grade.

REAGENTS

Preparation of reagents
Method-A

MBTH solution (Fluka; 0.2%, $8.56x10^{-3}$M): Prepared by dissolving 200mg of MBTH in 100 mL of distilled water.

$NaIO_4$ solution (BDH; 0.2%, $9.35X10^{-3}$M) : Prepared by dissolving 200mg of sodium meta per iodate in 100mL of distilled water and standardized iodometrically.

Acetic acid solution (Qualigens; 0%,v/v,3.49M): Prepared by dissolving 20mL of glacial acetic acid to 100mL with distilled water.

Method-B

AV Solution (Loba; 5%, 2.618 x 10^{-2} M): Prepared by dissolving 5gms of ammonium vanadate in 100mL of distilled water.

Conc.H_2SO_4 (Qualigens): Used as it is.

Preparation of standard drug solution:

Milnacipran (pure) (100mg) was accurately weighted and dissolved in distilled water transferred to a standard 100mL volumetric flask. The final volume was made up to the mark with distilled water. The final concentration was brought to 250µg.mL^{-1} (**Method-A**) and 125µg.mL^{-1} (**Method-B**) respectively.

Assay of Milnacipran pharmaceutical dosage forms:

Ten tablets of the Milnacipran drug were weighed and powdered, and a quantity of the powder equivalent to 100mg was dissolved in 25.0mL of methanol shaken well and filtered. The filtrate was diluted to 100mL to get 1mg/mL solution of drug in formulations. The general procedure was then followed in the concentration ranges mentioned above for the assay of Milnacipran.

Method-A

Aliquots of standard Milnacipran solution (0.5-2.5mL, 250µg.mL^{-1}) were accurately measured and transferred into a series of 10.0mLvolumetric flasks. 3.0mL of MBTH solution, 1.5mL 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 650nm against a reagent blank. The amount of MCN present in the sample was computed from the calibration graph.

Method-B

Delivered aliquots of standard Milnacipran solution (0.5-2.5mL, 125µg.mL^{-1})) were delivered in to a series of 25.0mL calibrated tubes. To each tube 1.0mL of 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 25.0mL with ethanol. The resulting absorbance was measured at 740nm against a reagent blank. The amount of Milnacipran was computed from to appropriate calibration graph.

RESULTS AND DISCUSSION:

Spectral Characteristics:

In order ascertain the λ_{max} (optimum wavelength of maximum absorption) of the colored species formed in each of two spectrophotometric methods, specified amounts of Milnacipran (40 µg.mL^{-1} for Method-A and 20 µg.mL^{-1} for Method-B respectively) in final dilution were taken and the colors were developed separately following above mentioned procedures individually. The absorption spectra were scanned on a spectrophotometer in a wavelength region 300-780nm against a corresponding reagent blank. The reagent blank absorption spectrum of each method was also recorded against appropriate solvent.

Optical Characteristics:

In order to test whether the colored species formed in the methods (A&B) adhere to Beer's law the absorbance at appropriate wavelength of a set of solution containing different amount of Milnacipran and specified amount of reagents were noted against appropriate reagent blanks. The Beer's law was obeyed for both the methods (A&B). Least square regression analysis was carried out for the slope intercept and correlation coefficient. Beer's law limits molar absorptivity, and Sandell's sensitivity for Milnacipran with each one among mentioned reagents were calculated. The optical characteristics are presented in Table – 1.

TABLE-1: QUANTITATIVE PARAMETERS AND PRECISION DATA

Parameter	Method-A	Method-B
λ_{max} (nm)	650	740
Beer's law limits (μg/mL)	5.0 – 25.0	2.5 – 12.5
Detection limit (μg/mL)	0.6921	0.2354
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	2.45×10^3	7.88×10^3
Sandell's sensitivity(μg.cm^{-2}/0.001 abs. unit)	0.0926	0.030
Optimum photometric range (μg/mL)	6.0 – 15.9	3.6 - 7.5
Regression equation (Y=a+bc) slope (b)	0.0099	0.032
Standard deviation on slope (S_b)	1.38×10^{-4}	3.63×10^{-4}
Intercept (a)	0.0024	0.0044
Standard deviation on intercept (S_a)	2.29×10^{-3}	2.51×10^{-3}
Standard error on estimation (S_e)	2.19×10^{-3}	2.39×10^{-3}
Correlation coefficient (r)	0.9997	0.9998

Nature of colored species:

Method – A: In this reaction, Milnacipran gives oxidative coupled product with MBTH in the presence of an oxidant. Under the reaction condition MBTH looses two electrons and one proton during oxidation forming 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 in the benzene ring of the coupler. The probable sequence of the reactions is presented in scheme given below (Scheme-I) which exhibiting λ_{max} at 650nm.

Method-B: In this method the reduced form of Fe (III) (i.e. Fe (II) has a tendency to give a colored complex on treatment with $[Fe(CN)_6]^{3-}$. (Scheme-II) which exhibiting λ_{max} at 740nm.

Analysis of formulations

Commercial formulations containing Milnacipran were successfully analysed by each proposed method. The values obtained by the proposed and reference methods for formulations were compared statistically by the t- and F- tests found not to differ significantly. Recovery studies were conducted by analyzing each pharmaceutical formulation in the first instance for the active ingredient by the proposed methods. The results are incorporated in Table-2.

TABLE -2: ASSAY OF MILNACIPRAN IN PHARMACEUTICAL FORMULATIONS

Formulation*	Amount taken (mg)	Amount found by proposed Methods**		Reference method	Percentage recovery by proposed Methods***	
		Method-A	Method-B		Method-A	Method-B
Tablet - I	25	25.09±0.13 F=3.69 t=0.82	24.89±0.12 F=4.34 t=0.18	24.91±0.25	100.40±0.18	99.95±0.17

* Tablet from one pharmaceutical company.

** 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 proposed two methods were found to be simple, selective and sensitive. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the authentic samples containing Milnacipran showed no interference from the common excipients. Hence, these methods could be considered for the determination of Milnacipran in the quality control laboratories.

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