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GREEN CHEMISTRY: AN ECO-COMPATIBLE APPROACH FOR NANODROP SPECTROPHOTOMETRIC DETERMINATIONOF AMITRIPTYLINE IN PURE AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

This paper describes a new spectrophotometric determination of an antidepressant drug Amitriptyline which is most commonly used to treat the symptoms of depression. The molecular formula for Amitriptyline HCl is $C_{20}H_{23}N.HCl$. Iron-thiocyanate complexation method forms the basis of the present research work. The absorbance of the series of blood red colored Iron-thiocyanate-drug complex thus developed was measured at λ_{max}490 nm against the reagent blank using the sophisticated Nano-drop spectrophotometer instrument which requires only micro-drop of samples and thus, serves as a green technique involving much lesser use of solutions for sample preparation and analysis. The results show that Beer"s law was verified for the range of the Iron (III) concentration studied. The method is found to obey Beer's law at the concentration range of 0.5-5.0 mg/L with slope, intercept and correlation coefficient 0.19063, 0.012 and 0.99665 respectively. The limit of detection of the method is found to be 0.0038 mg/ L.Optimization of the method was performed considering various parameters. This novel method is successfully applied for the determination of Amitriptyline in single drops of samples using NDS and results are compared with the previously developed methods.

Keywords: Pharmaceuticals, Tricyclic anti-depressants, Amitriptyline, Nanodrop spectrophotometer; Green-technology.

INTRODUCTION

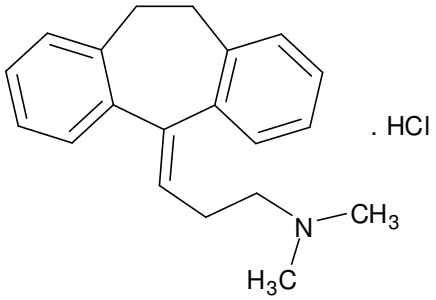
Amitriptyline belongs to tricyclic class of antidepressant drugs and is metabolized by demethylation to a more potent selective norepinephrine reuptake inhibitor Nortriptyline[1].Amitriptyline was first discovered in the year 1960 and approved by US Food and drug administration (FDA) in the year 1961.It is majorly used to treat a number of mental disorders including major depressive disorders and anxiety disorders[2]. Other uses are also reported as in neuropathic pains, insomnia, migraines, anxiety disorders and as an anticholinergic drug[3].The function of amitriptyline is to block reuptake of the neurotransmitters: serotonin and norepinephrine in the central nervous system. Apart from its therapeutic effect it may cause side effects such as: convulsions, paresthesia, hallucinations, delusions, tachycardia or, arrythmia[4]. Therefore, it is required to prescribe the proper therapeutic dose of the drug for efficient treatment.

Amitriptyline hydrochloride is chemically known as 3-(10, 11-dihydro-5H-dibenzo [a, d] cycloheptene-5-ylidine)-N, N-dimethyl-1-propanamine hydrochloride and its structure is shown below in Table-1. Amitriptyline is official in IP, BP and USP.[5]

Literature review reveals that several methods are reported for analysis of Amitriptyline including AASspectrophotometric[6-11,] spectroflurimetric,chromatography,[12] flow injection method and UV-Visible spectrophotometric methods[13]. Gendy et al. in 1993 have also reported determination of Amitriptyline and other drugs of central nervous system.[14] However, most of these methods are either tedious or time consuming or, are very sophisticated and generally not available in routine laboratory and involves large amounts of samples for analysis. Also these analyses are often accompanied with the presence of excipients especially in coated Tablet forms which leads to difficulties like presence of coloring matter and other additives[15].Spectrophotometric determinations are the most commonly adopted analytical

methods due to their simplicity, low cost and common access to the apparatus however, it suffers from many limitations specially sensitivity and selectivity problems[16].

Table-1: Chemical structure and pKa of Amitriptyline. HCl

Name	Molar mass	Chemical structure	pKa
Amitriptyline. HCl	313.86 g/mol	 Amitryptyline.HCl	9.76

Thus, this method involving Iron-thiocyanatecomplexation and analysis using NDS is developed which minimizes the use of chemicals and solvents for sample preparation thus serving as simple, fast and sensitive method. Various parameters such as concentration and volume of acid are also considered for the optimization of the proposed method[17].Further this method doesn't require heating and other pre-extraction steps and can be easily applied for routine analysis of amitriptyline and other such drugs in pharmaceutical laboratories. Hence in the present communication we propose a novel eco-compatible technique using Nanodrop spectrophotometer for the analysis of amitriptyline in pure and pharmaceutical dosage forms.

EXPERIMENTAL

Materials and Reagents

All the chemicals and reagents used were of analytical reagents AR grade. The working solution of iron was prepared by diluting the standard solution containing 1.000 g Fe (III) / L with deionized doubled distilled water to an appropriate dilution. Working solutions of ammonium thiocyanate(1.0 M) was prepared by dissolving 9.5 g of ammonium thiocyanate in 50.0 ml of double distilled water taken in a 50 mL volumetric flask and further diluted to appropriate concentration.NaOH (0.1 N) and HCl (0.1 N) for pH adjustment were also prepared using deionized double distilled water. Amitriptyline.HCl (MW=313.86) was purchased from Sigma (St. Louis, MO, USA). All the solutions were mixed in a 2.0 ml vials before analysis.

Standard solution of Amitriptyline

A 1000.0 ppm solution of amitriptyline HCl was prepared by dissolving 10.0 mg of the drug into a 10.0 mL volumetric flask using deionized double distilled water. Further dilutions of the stock solution were made to prepare 100.0 ppm solution which was further diluted to prepare working solutions of range 0-5.0 mg/L in 2.0 ml vials.

Instrumentation

ThermoscientificNanodrop™1000 spectrophotometer was employed for the measurement of absorbance using 1.0 microliter of samples. Patented sample retention technology that employs surface tension alone to hold the sample in place is used. This eliminates the need for using large volume of samples in cuvettes as in traditional UV-VIS spectrophotometric technique and also allows for cleanup in seconds. The instrument measures 1.0 µl of sample with a high accuracy and reproducibility. LI-617 pH meter (Elico, Hyderabad, INDIA) was used to maintain the pH of the solution before analysis.

Procedure

In each of the 2.0 ml vials, 200.0 µl of standard 10.0 mg/L Iron(III) solution and 800.0µl of 1.0 M ammonium thiocyanate were pipetted out using a thermo-scientific micropipette (20-200 ul range). 200 µL of 0.1N HCl was added to each vial to adjust the pH to appropriate level. Then, AmitriptylineHCl solution having varying concentration from 0 – 5.0 mg/mL was added to the vials and the volume was made upto 2.0 ml in each vial using deionized double distilled water. Absorbance was measured at 490 nm against the reagent blank and a calibration curve is plotted between absorbance and varying concentrations of the drug.

RESULTSAND DISCUSSION

In this method the negatively charged thiocyanate complex (anion) of Fe(III) binds electro statically with positively charged amitriptyline (cation) forming a blood red colored complex in acidic solutions with an absorption maxima at 490 nm against reagent blank and a calibration curve is plotted by using Beer-Lamberts law (y=mx+c).

$$Fe^{3+}(aq) + n\,SCN^{-}(aq) \longrightarrow [Fe(SCN)_n]^{3-n}(aq) \quad \text{(Blood Red Colored)} \quad (1)$$

Iron-thiocyanate complex

$$Fe^{3+}(aq) + n\,SCN^{-}(aq) + m\,(AMI)^{+}(aq) \longrightarrow [Fe(SCN)_n]^{3-n} \cdot m\,(AMI)^{+}(aq) \quad (2)$$

Iron-Thiocyanate- Ami complex

Where, the value of n varies from 2 to 6 in acidic media.

The increase in absorbance on addition of the drug may be attributed to the hyperchromic shift[18]. The precision of the method was studies using interday and intraday precision.

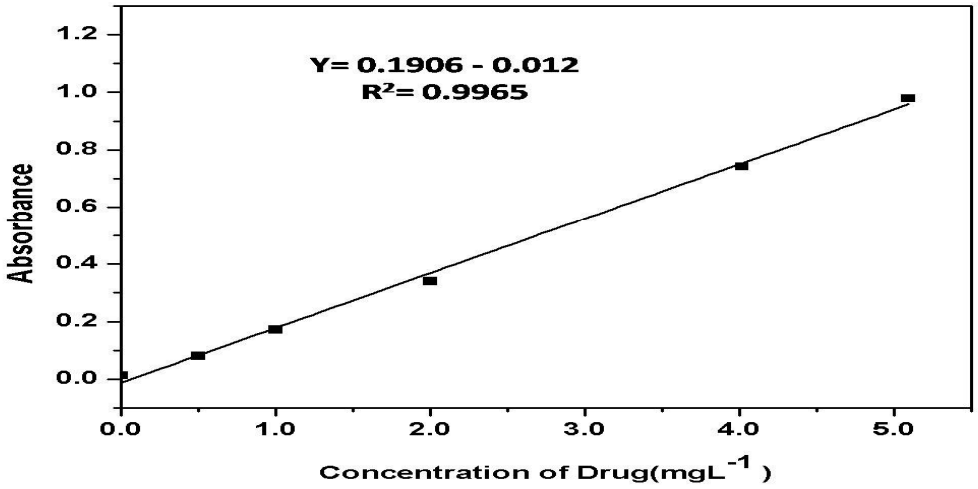


Fig.-1: Absorption spectra of the blood red Fe (III)-SCN at λmax 490 nm under optimized conditions (at pH 3).

Optimization of the variables

a. Effect of Iron(III) concentration

During the optimization of analytical variables concentration of iron (III) solution was varied from 0.5-4.0 mg/L. Maximum and stable absorbance was obtained at 2.5 ppm iron (III) concentration and was thus chosen for the development of iron-thiocyanate complex.

b. Effect of Thiocyanate concentration

The effect of thiocyanate concentration on the formation of iron-thiocyanate complexation was examined by varying the thiocyanate concentration from 0.5-2.5 mg/L in the aqueous phase. It was found that

increase in thiocyanate concentration causes an increase in complex formation. However, the complex showed maximum absorption at 1.0 M concentration of ammonium thiocyanate.

c. Effect of various acids

Effect of different acids like HNO_3, HCl, H_2SO_4 was also tested[19]. Use of HCl for maintaining pH was less efficient which may be due to formation of mixed ligand ions. H_2SO_4 gave the best result and was thus chosen as a medium for making Fe-thiocyanate complex. The concentration of H_2SO_4 was varied from 1.0- 9.0 M. But relatively low absorbance values of the complex at higher acid concentration is observed which may be attributed due to hydrolysis of iron (III) ions in solutions.

d. Effect of pH

The pH of the sample solution is known to play an important role in the determination of tricyclic antidepressant drug. The drug Amitriptyline is having a pK_avalue of 9.1. In the present investigation, a wide range of pH of the solution was adjusted from 2 to 12 with the addition of 0.1 N HCl and 0.1 N NaOH solutions.The absorbance of the red colored complex increases with the increase in pH but it was found that the red colored complex turned colorless at pH 6 and above. Therefore the pH of the sample solution was maintained at 3.0 throughout the experiment.

Comparison of the method

The present method is found to be appropriate keeping in view the idea of green technology. The method consumes only few micro-liters of the samples for analysis using NDS and the reagents required are also comparatively cheaper and easily available in common routine chemical laboratories. The analysis time is also lesser as compared to the other methods reported till date [20-24], shown in Table- 3. The analytical performance of the proposed method is validated according to the provided guidelines.The main advantages of the method is low cost of reagents used and short analysis time also, no interference in the measurements of absorbance was observed. Further, this method using Fe-SCN complex can be applied at ambient temperature and color development is instantaneous.

Sensitivity and statistics

The calibration curve is obtained by plotting absorbance versus concentration of the drug. The method follows Beer's law over the concentration range of 0.5 to 5.0 mg L^{-1} for the drug. Slope, intercept and correlation coefficient values are 0.19063, 0.012 and 0.99665 respectively, shown in Table- 3. The LOD of the method is found to be 0.0038 mg/L. The maximum absorption values for the drug is obtained at λmax 490nm.

Table- 2: Comparison of the present method with previously reported methods for the analysis of Amitryptyline.HCl.

Number	Methodology	Reagents used milliliter	Linear Range Microgram per milliliter	Limit of detection Nanogram per milliliter	Reference
1.	UV-Visible Spectrophotometry	Ammonium molybdate	1-140	1.0	22
2.	Dispersive liquid-liquid microextraction	Carbon tetrachloride and methanol	0.005-16.0	0.005	23
3	Capillary zone electrophoresis	Tris-buffer and ß-cyclodextrin	0.16-0.63	0.06	24

Application of the present method

The present work finds extensive application in the field of real time analysis of drugs as in case of pharmacokinetics when coupled to GC-MS analysis. To investigate the validity of the proposed method, 25 µ L of the human blood samples from psychiatric patients with informed consents were spiked with known concentration (10 and 250 ng/mL) of the drug and the calculated recoveries were well within the precision level. Further, applicability was supported by the present method using the eco-compatible NDS method and the recovery range was found to be 94-97% with corresponding RSD values from 1.8 -1.9, shown in Table- 4. This method can also be extended in cases of forensic sciences to determine the concentration of drugs (drugs of abuse) in cases where the samples available are usually scarce. The method could be easily coupled to micro-extraction method of sample preparation for the analysis of other pharmaceutical drugs from biological samples like blood, plasma, saliva, hairs etc.

Table- 3: Determination of linear range, coefficient of correlation, Limit of detection and Relative standard deviation % in blood sample.

Drug	Linear range (milligram per Liter)	Coefficient of correlation	Limit of detection (nanogram per milliliter)	Relative standard deviation (%)
Amitryptyline	0.5-5.0	0.996	3.8	1.4

Table- 4: Application in blood samples for calculating recovery percentage

Drug	At 10 nanogram per milliliter		At 250 nanogram per milliliter	
	Recovery (%)	Relative standard deviation (%)	Recovery (%)	Relative standard deviation (%)
Amitryptyline	94.0	1.8	97.0	1.9

CONCLUSION

In this study a simple, rapid, sensitive, accurate and precise Nanodrop spectrophotometric method for the determination of amitriptyline hydrochloride in bulk and pharmaceutical formulation has been developed and validated. It was found that the common excipients present in theformulation did not interfere with the proposed method and could be used for the routine quality control analysis of amitriptyline hydrochloride in bulk as well as in pharmaceutical dosage forms or, marketed capsules. This study is of interest for forensic toxicologists to determine the concentration and effects of the drug in small volume of biological samples ranging from blood, serum, plasma and urine samples by coupling it to micro-extraction techniques. Thus, this method exhibits many advantages such as simplicity, short analysis time, minimum consumption of sample solution and solvents etc.

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