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DEVELOPMENT AND VALIDATION OF A METHOD FOR QUALITATIVE & QUANTITATIVE ANALYSIS OF AMITRIPTYLINE USING UV VISIBLE SPECTROPHOTOMETER

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ABSTRACT

Amitriptyline is a tricyclic antidepressant, anticholinergic drug and has sedative properties, which is mainly used for treating depression. On overuse of amitriptyline, dizziness, headache, weight gain, delirium, disorientation, anxiety, and agitation are some of the side effects. This drug has many side effects and causes high toxicity in case of overdose. Deaths due to toxicity of amitriptyline are relatively common. It raises the risk of suicidal thinking and behavior in people of all age groups. The adverse effects increase the drug's medico-legal importance and hence it becomes necessary to analyze this drug for identification purpose in the field of forensic analytical toxicology. Routinely, High Performance Liquid Chromatography, Gas Chromatography, Gas Chromatography-Mass-Spectroscopy and Liquid Chromatography-Mass Spectroscopy are used for analysis of amitriptyline. These techniques are not only costly but also require more sophisticated instruments which are not available in each laboratory. In this study, analysis was performed by UV-Visible spectrophotometer in pure formulation on the basis of absorbance of UV absorption by target compound. A simple, precise and highly cost efficient UV spectroscopic method has been formed for the analysis of amitriptyline. The technique was validated according to the United States Pharmacopoeia (USP) guidelines, which looked at the technique's range, linearity, accuracy, recovery, precision, and sensitivity. In Distilled Water: Methanol (9:1)v/v, amitriptyline had a

maximum wavelength of absorbance of 240 nm and obeyed linearity or Beer-law Lambert's in the concentration range of 5-25 g/ml. The regression coefficient (R^2) was determined to be 0.997. 3.1910 g/ml and 9.6697 g/ml were determined to be the Limits of Detection (LOD) and Quantification (LOQ), respectively. The new approach was repeatable, cost-effective, and non-destructive, allowing it to be used effectively for the accurate and precise analysis of amitriptyline.

KEYWORDS: Method Development, USP validation, Amitriptyline, UV-Vis spectroscopy, Linearity.

INTRODUCTION

Amitriptyline is a derivative of dibenzocycloheptadiene, chemically IUPAC name:3-(10, 11-dihydro-5*H*-dibenzo [*a*,*d*]cycloheptene-5-ylidene)-*N*,*N*-dimethylpropan-1-amine, tricyclic antidepressant drug that is commonly used to treat depression. It possesses sedative and anticholinergic effects. It appears to block the re-uptake of norepinephrine and serotonin at nerve terminals, enhancing the effects of these neurotransmitters, as well as antagonising cholinergic responses to bioactive amines.[1] Amitriptyline is a rare cause of clinically evident acute cholestatic liver damage and can induce modest and temporary blood enzyme increases.[2] Amitriptyline was initially synthesised in 1960[3], and it was initially used for medicinal purposes in the United States in 1961[4] under the brand name Elavil. It is rapidly absorbed via the GI tract and substantially metabolised into nortriptyline by CYP2D6, CYP3A4, and CYP2C19-mediated N-demethylation.[9] Amitriptyline is a tertiary amine, whereas nortriptyline, its side chain demethylated metabolite, is a secondary amine[5-6], a more powerful and selective norepinephrine reuptake inhibitor, may complement its action on norepinephrine reuptake.[9] Amitriptyline has an elimination half life duration of 25 hours and its volume of distribution is 10-50 ml/kg.[10]

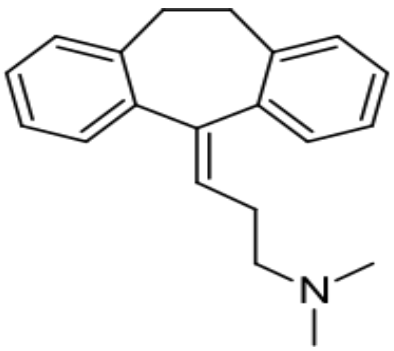

Figure No. 1: Showing structure of Amitriptyline.

Amitriptyline has a number of side effects, the most frequent of which are dizziness, headache, and weight gain, which affect more than 1% of users. Delirium and disorientation are common cognitive adverse effects, as well mood changes including anxiety and agitation. Cardiovascular adverse effects include orthostatic hypotension, sinus tachycardia, and QT-interval prolongation.[12] Drowsiness, Hypothermia (low body temperature), Tachycardia (fast heart rate), Other arrhythmic abnormalities such as bundle branch block, Congestive heart failure, Dilated pupils, Convulsions (e.g. seizures), Severe hypotension (very low blood pressure), Stupor, Coma, and even death are all possible symptoms of amitriptyline overdose.[8]

Other adverse effects include tiredness, sleeplessness, and nightmares, as well as sexual adverse effects such as loss of libido and impotence. Amitriptyline is considered to have the greatest anticholinergic adverse effects and to be the most prone to cause delirium of all of the TCAs.[13]

Overdose symptoms and treatment are generally the same as for the other TCAs, including the appearance of serotonin syndrome and serious cardiac consequences. Because amitriptyline and other TCAs are no longer indicated as first-line therapy for depression, according to the British National Formulary[14], they are no longer indicated as first-line therapy for depression. Nick Drake, an English folk singer, died in 1974 from an overdose of Tryptizol.[15]

Suicidability and Antidepressant Drugs

By the regular use of Antidepressants, the chances of suicidal ideation and behavior (suicidality) increases in relation to short-period studies of psychiatric disorders and other major depressive disorder (MDD). The risk of intake of Amitriptyline hydrochloride tablets must be balanced with the clinical need. The usage of this substance may intensify the effects of alcohol as well as barbiturates and other CNS depressants. It is not recommended for usage in children. The effects of drug on the patients differ accordingly based on their history and other bodily factors. Amitriptyline or doxepin are usually with high sedative effects compared to protriptyline.[16] ***Jicks SS et al.1995*** evaluated the risk of suicide of 8.5/10000 person/year and violence 15% and overdose is 35% of drug were the major methods used for the suicide respectively.[26] ***Olgunet al. 2009*** studied effect of AMT poisoning in relation ECG clinical status in 52 poisoning cases of AMT. Result shows poisoning can result in ECG changes. ***Paksu S et al. 2014*** examined 250 patient with acute AMT poisoning.[28]

METHOD DEVELOPMENT AND VALIDATION[17]

Method Development

Analytical methods are being developed in order to establish a precise assay approach for determining the composition of distinct substances. They must be prepared in accordance with the ICH Q2 (R1)/USP guidelines' methodologies and acceptance criteria.

The following are the steps of developing a method

1. Characterization of standard analyte, Method requirements, Searching Literature, Selecting the method, Instrumental setup and preliminary study, Optimization of parameters, Documentation of analytical figure, Analysis of the developed method with the sample, Determination of percent recovery of the sample, Demonstration of quantitative sample analysis.

Step for Validation of the method

Typical validation parameters recommended by FDA, USP and ICH are as follows

1. Specificity
2. Linearity and Range
3. Precision

(A) Method precision (Repeatability)

(B) Intermediate precision (Ruggedness)

4. Accuracy
5. Solution stability
6. Limit of Detection (LOD)
7. Limit of Quantification (LOQ)
8. Robustness

The proposed approach has been verified according to USP requirements in the current study. The current study aimed to establish a simple and cost-effective approach for drug identification and quantification, as well as to validate the approach using UV-Vis spectroscopy in accordance with USP criteria.

Using a UV-visible spectrophotometer, an attempt was made to design and verify a technique for qualitative and quantitative analysis of Amitriptyline. As the use of this drug is having number of side effects and causes high toxicity even death on overdose. It raises the risk of suicidal thinking and behavior in individuals of various age groups.[16] Therefore all these

adverse effects this drug increase the medico-legal importance and hence it becomes necessary to analyze this drug for identification purpose in the field of forensic analytical toxicology.

MATERIALS AND METHOD

Materials

Year/experimentation site: 2019/ Forensic science and Toxicology Department, Chandigarh University, Mohali, India.

Sample Collection

Standard (100% pure) drug was received as gift sample from the Sigma- Aldrich, India.

Chemicals and solvents

For conducting the UV spectrometric method development study, ultrapure distilled water was obtained from distillation unit and methanol was procured from Active fine chemicals was used as solvents for the preparation of stock and working solution in the study.

Equipment's and Instruments

An UV-Visible double beam spectrophotometer (Shimadzu, Model-UV1900) with matched quartz cell of 1cm path length was used for experimentation. All weighing was done on electronic balance (Citizon, Model-CX220) and all solution were sonicated using a Sonicator (HWASHIN Power sonic 520) in the experiment.

METHOD

Preparation of stock solution

10 mg of Amitriptyline standard salt was weighed and transported to a 100 ml volumetric flask, where it was dissolved in a 9:1 solution of distilled water and methanol. Then volume was raised to 100 ml with the help of diluents and was sonicated for 10 minutes for better dissolution.

From the stock solution that has been prepared 25 mL of solution was placed in a 100 mL volumetric flask, and the volume was adjusted with diluents to the desired level (25 ppm). Methanol: distilled water (9:1 v/v).

Preparation of Serial Dilutions

Various solution concentrations 5ppm, 10ppm, 15ppm, 20ppm, and 25ppm stock solutions were generated using a solvent system from a 100ppm (100g/ml) stock solution. Methanol is found in distilled water (9:1). In order to achieve 5ppm, 0.5ml of stock solution was placed in a 10ml volumetric flask and the volume was increased using the solvent system. Similarly, for the 10ppm, 15ppm, 20ppm and 25ppm the amount of solution taken from the stock solution is 1ml, 1.5ml, 2ml and 2.5ml respectively in the 10 ml volumetric flask and the remaining volume is made up to the level with the diluents.

Determination of wavelength of maximum absorbance

To determine the maximum adsorption wavelength against blank, the above produced stock solution of AMT was scanned in the range 200-400 nm. The maximum wavelength of AMT was found to be at 240 nm. Therefore, 240 nm was selected as the λmax. for AMT.

Construction of calibration curve

The prepared concentrations 5ppm, 10ppm, 15ppm, 20ppm and 25ppm were used to prepare the calibration curve. Later, calibration curve was plotted between absorbance versus concentration to get the linearity and regression equation which was shown in fig.3.

The technique was validated using the United States Pharmacopoeia (USP) guidelines, which looked at the technique's range, linearity, accuracy, recovery, precision, and sensitivity.

RESULTS AND DISCUSSION

Maximum wavelength for AMT (λmax.)

Maximum wavelength for AMT was found to be 240 nm during scanning between 200-400 nm UV ranges.

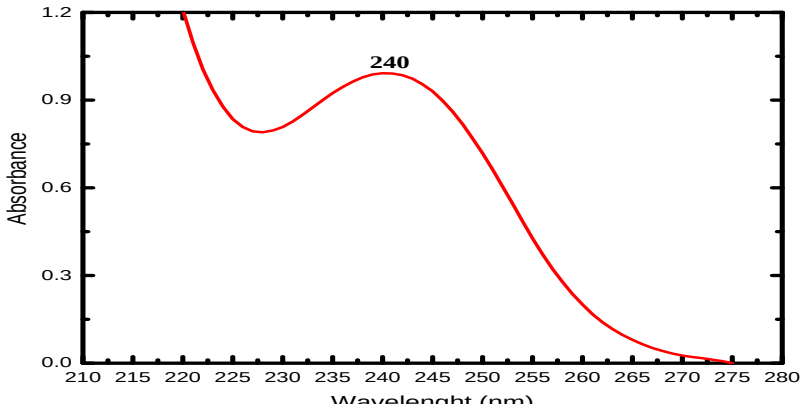

Fig. No. 2: Showing the maximum absorbance at 240nm.

4.2 Construction of Calibration Curve

Five standard solutions (5, 10, 15, 20, 25 g/ml concentration) were produced to generate the calibration curve. At a wavelength of 240 nm, the absorbance values of these solutions were recorded (max. wavelength for AMT). To obtain the standard curve, data was gathered and plotted on a chart. A regression co-efficient value (R^2) of 0.997 was calculated from this curve.

Table 1: Showing Absorbance data of standard solution of AMT at 240nm.

Conc.(µg/ml)	Absorbance
5	0.216
10	0.485
15	0.693
20	0.936
25	1.141

A straight line was formed by graphing the absorbance versus the concentration of AMT. The calibration curve yielded the equation Y=0.04602x+0.0039.

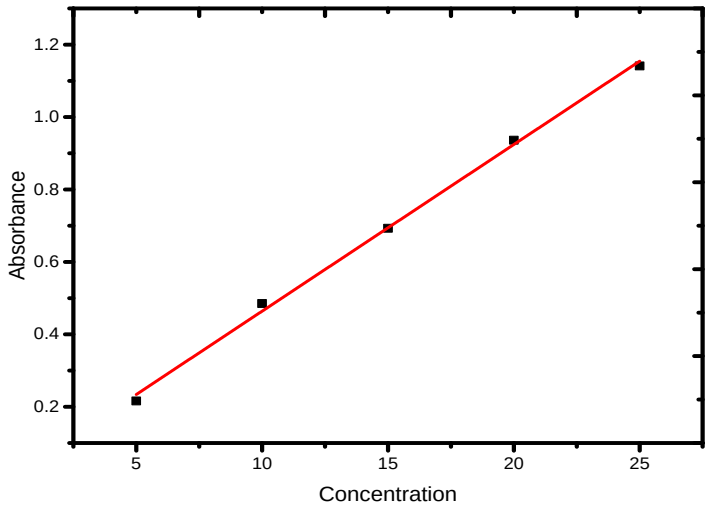

Fig. No. 3: Showing Calibration Curve of AMT in Distilled Water: Methanol (9:1) at 240 nm.

Method Validation Parameters

Linearity

The linearity was evaluated by analyzing the calibration curve of AMT. The drug's response was found to be linear in the study, with a concentration range of 0.04602x+0.0039 and a linear regression equation of Y= 0.04602x+0.0039 with a correlation coefficient of 0.997 and a correlation coefficient of 0.998.

Range

The method followed the Beer-Lambert's law from the concentration range of 5-25 µg/ml.

Precision

Three independent replicates at three distinct concentrations (n=3) were used to measure precision, which was quantified as relative standard deviation.

Here, we examined the differences in UV absorbance of AMT solutions at different time in a particular day and at different days. To do so 3 sets of 5, 10 and 15 µg/ml solutions were made from 100ug/ml stock solutions and were analyzed in the morning and afternoon and then for the 3 consecutive days respectively.

Sensitivity of the method

The suggested approach used a calibration standard to calculate the LOD and LOQ of Amitriptyline. The standard deviation of the blank response, standard deviation of the ordinate intercept, or residual standard deviation of the linear regression were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the standard calibration curve and is the standard deviation of the blank response, standard deviation of the ordinate intercept, or residual standard deviation of the linear regression.

Recovery/Accuracy

Recovery studies were carried out to confirm the dependability of the suggested approach. AMT was introduced at 5ppm, 10ppm, and 15ppm levels to an equal ppm of medication of known amount, and the content was reanalyzed using the suggested approach. The method recovered 86.7-91.6% of the spiked amount of AMT in a sample solution. This showed that the method of analysis could be applied to analyze drug in the presence of interfering substances. The recovery is close to spiked amount 100% showed that metric does not interfere in the analysis. The percentage loss was found due to handling errors while recovery. The percentage lose is acceptable in recovery of drug.

Table 2: Showing recovery data of Amitriptyline.

Metric	Amt. of standard spiked	% recovery	Mean	SD	%RSD
Blood	5ppm	90.8	89.7	0.78	0.87%
	10ppm	86.7		2.12	2.36%
	15ppm	91.6		1.34	1.49%

Table 3: Showing summary of the method validation parameters.

Parameters	Values
Λmax.	240nm
Beer's limit	5-25µg/ml
Reg. equation	Y=0.04602x + 0.0039
R^2	0.997
Slope	0.04602
Precision (Interday) 1st day	2.39%
2nd day	5.58%
3rd day	2.39%
Accuracy	86.7-91.6%
LOD	3.1910µg
LOQ	9.6697µg

Summary of the method validation parameters

The total method validation parameter data is summarized in the following table No. 3.

DISCUSSION

The development of analytical techniques for determining drug concentrations has been very beneficial in the recent years as these methods assures the quality of the drug products and used for the purpose of identification in cases related to poisoning/overdose in the field of forensic science. The objective of this research was to come up with a quick and low-cost UV spectrophotometric technique for estimating Amitriptyline. Distilled water and methanol in a 9:1 ratio were chosen as the solvent system for the technique development after evaluating solubility and stability. The maximum absorption wavelength found for AMT in current study was 240nm where as 239nm is also reported in some other researchers[18,21-22, 24] moreover some found maximum absorption at 222nm[23] and 219 nm[25] of wavelength.

It obeys Beer- Lambert's law in the concentration range of 5 – 25 µg/ml and used for linearity and validate according to USP guidelines, where several ranges were used by other researchers as 0-15 µg/ml[20], 2-20 µg/ml[23] and 5-17 µg/ml[25] and verified according to ICH guidelines.

The optical properties, such as percent relative standard deviation and percent range of error, were determined to be acceptable and within the limits. The average RSD for intraday precision and interday precision is 3.19 and 3.45 respectively which is in a recommended range. The results revealed that the approach has a respectable degree of precision, and numerous research have shown that the smaller the computed RSD, the more exact the measurement. Current method shows good recovery index which is supported by previous

studies by **Patel *et al. 2010***, **Deepakkumari HN*et al. 2013* &Rambabu C *et al. 2014***[19-20,22] and USP standards. The suggested method's analytical validation parameters were all derived using USP recommendations.[10] The findings of all validation parameters were within the range of USP standards.

The accuracy of the procedure was proven by recovery trials, which involved introducing a known amount of pure medication to the matrix (blood) and calculating the % recovery. The LOD and LOQ were found to be very low, confirming the method's sensitivity, and it may be used for routine Amitriptyline analysis or in situations of toxicity or overdose.

CONCLUSION

A very sensitive, accurate, precise, and cost-effective spectrophotometric approach for determining Amitriptyline in pure drug form or for analysis in situations of toxicity/overdose is presented in this study. The proposed approach complies with USP standards. The statistical analysis revealed that the data from the suggested approach for determining Amitriptyline is in excellent agreement. It does not require any expensive experimental set up like HPLC, other chromatographic techniques and any other higher instrumentation. The approach is free of any common analytical complications, therefore, it may be easily used for regular quality monitoring of Amitriptyline in conventional laboratories.

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Conflicts of Interest: Author shows no conflict of Interest with this manuscript.

Abbreviations, Units Etc:

AMT: Amitriptyline

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