ORIGINAL RESEARCH

STABILIY INDICATING ASSAY OF AMITRYPTILINE IN PHARMACEUTICAL DOSAGE FORM BY CHEMICAL DERIVITISATION METHOD WITH EBT INDICATOR USING UV VSIBLE SPECTROPHOTOMETER

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ABSTRACT:

A simple, accurate, precise, validated uv-visible spectrophotometric method is developed for quantitative determination of tricyclic antidepressant drug, Amitryptiline (AMT) in pharmaceutical formulations. The indirect spectrophotometric analysis is optimized by chemical derivatization method for better analysis of selected drug. The Eriochrome Black T indicator which undergoes diazotization reaction with tertiary amines is selected and shown absorption in the range of 400-800nm.Beer's law is obeyed (concentration range of 1-10 µg/mL) and validated for linearity, accuracy, precision and robustness. The drug was subjected to stress degradation under different conditions recommended by ICH to study the stability of drug. The proposed method was successfully applied to analysis of commercial tablets containing amitryptiline and the results were in under acceptance criteria with those obtained with reported works.

KEYWORDS: Amitryptiline, chemical derivatization, UV-VIS spectrophotometer, stability studies, ICH guidelines

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INTRODUCTION

Chemical derivatization falls under the category of indirect spectrophotometric analysis, which welcomes the shift of the absorption spectrum to colorimetric range for analysis of weakly absorbing analytes and is common with most drugs, including interference of solvents, excipients and extraneous matter along with main active ingredient, where there is a need to improve selectivity of the procedure or sometimes cost implications will favor adoption of a colorimetric method to that of a uv –vis spectrophotometer. Forced degradation studies help to know the formulation process impurities, degradation products formed during different stress conditions like acid, alkali, neutral, oxidation and photolytic by giving an idea of stability, therapeutic efficacy and safety of the drugs.

Amitryptiline chemically 3-(5,6-dihydrodibenzo[2,1-b:2',1'-f][7]annulen-11-ylidene)-N,N-dimethylpropan-1-amine is a tricyclic antidepressant drug, which is metabolized to nortriptyline and inhibits the reuptake of norepinephrine and serotonin almost equally, which may contribute to the antidepressant activity.

Various analytical methods have been reported for the antidepressant drug Amitryptiline Hydrochloride (AM) including spectrophotometric, chromatographic techniques but have its own merits and demerits of performing them in laboratory conditions. Therefore, the main aim of the study includes chemical derivatization analysis of amitryptiline drug with selective reagent Eriochrome Black T (EBT) indicator along with stability studies.

MATERIALS &METHODS:

Eriochrome black T reagent is purchased from Finer Laboratories. Hydrogen peroxide, Hydrochloric acid, Sodium hydroxide from Lobachemie laboratories. The instrument used to carry out the work is ELICO SL-244 UV-VIS spectrophotometer. The amitryptiline tablets manufactured by Wockhartd Limited are purchased from local pharmacy.

METHOD DEVELOPMENT:

❖ *Selection of solvent*

According to literature available, as drug were easily soluble in water, methanol, moderately soluble in organic solvents. Here in this study the drug concentrations have been prepared using water as a solvent.

❖ *Selection of Wavelength Range*

The sensitivity of spectrophotometric method depends upon the proper selection of wavelength that gives maximum absorbance and good response for the given drug. The selected drug formulation of different concentrations has absorption at 201nm which may interfere with presence of excipients. Therefore to increase the absorption maximum suitable chemical derivatization technique is adopted which increases drug absorption range in between 400-800nm.

❖ *Selection of Reagent*

If the analyte absorbs weakly in the uv region, a more sensitive method of assay is obtained by converting the substance to a derivative with more intensely absorbing chromophore. In this study, the drug selected contains a tertiary amine group which on reaction with suitable reagent forms a coloured complex. Trials have been done using reagents like Ehrlich's reagent, DNPH reagent, EDTA, EBT indicator. The EBT indicator reacts with tertiary amine group forming coloured azo dye complex and it is found to be more stable and showed good

absorption maximum at visible range. So, EBT indicator is selected as our reagent.

❖ ***Procedure for calibration curve***

Various aliquots of standard solution containing 0.0, 0.1, 0.5, 1.0, 1.5, 2.0, 3.0mL of AMT (10µg/mL) were transferred into a series of 10mL calibrated flasks using standard pipette. To each flask 1.0mL of 0.1% EBT reagent was added and mixed well before diluting to 10mL with distilled water and absorbance of colored solution is measured at scanning wavelength range of 400-800nm against the reagent blank. The reagent blank was prepared similarly but without AMT.

❖ ***Preparation of sample solution***

Weighed 20 tablets and triturated to a fine powder. An amount of powder equivalent to 10mg of drug into a 100mL standard volumetric flask, dissolved in 50mL of deionised water. The volume was diluted to the mark with water and mixed well and filtered using a Whatman No. 41 filter paper. The filtrate containing the drug was at a concentration 100µg/mL was subjected to analysis by the procedure described above after suitable dilution step.

Solutions of working range concentration were prepared by proper dilution of this stock solution with water and constructed calibration curve of different concentrations.

❖ ***Preparation of Reagent***

Eriochrome black T indicator is easily soluble in water. Therefore 0.1% EBT reagent is prepared by dissolving 0.1mL EBT reagent in 100mL distilled water.

VALIDATION PARAMETERS:

Linearity: A stock solution was prepared by dissolving 10mg of the drug in 100ml of deionised water. Then from this stock solution dilutions of various concentrations from 0.1µg/ml to 3µg/ml were prepared. Each dilution was analysed in series, absorbance were observed to construct the standard calibration curve.

***Accuracy*:** Accuracy was determined by calculating recovery % of amitryptiline by standard addition method. The pre- analyzed sample solutions (1 µg/ml) were spiked with reagent concentration at three different levels- 50% (0.5mL), 100% (1.0mL), 150% (1.5mL). The resulting mixtures were reanalyzed using the proposed method. The experiment was conducted in triplicates. Accuracy was reported as % recovery.

***Precision*:** Precision of the proposed method was calculated by conducting intermediate precision.

1. Repeatability or Intra Day Precision: The intraday precision was determined by estimating the absorbance of three different concentrations of 0.5, 1.0, 2.0 µg/ml three times on the same day.

2. Intermediate or Inter Day Precision: Intermediate precision (inter day) was established by analysing the three different concentrations of 0.5, 1.0, 2.0 µg/ml on three different days and with different analyst on different days. The standard deviation, % relative standard deviation and estimated concentrations based on standard curve were reported for each set of data.

***Robustness*:** Robustness of the developed method was determined by injecting three different concentration (0.5, 1.0, 2.0 µg/ml) of drug and also changing the reagent concentartions in triplicates by varying the wavelength (±2). Robustness is reported in %RSD.

***LOD and LOQ*:** Detection limit and Quantitation limit of the drug is calculated by using the calibration curve standards. DL and QL was calculated from the equation 3.3σ/S and 10σ/S respectively, where σ is the standard deviation of y-intercept and S is the slope of the calibration curve.

RESULTS & DISCUSSIONS

1. CHEMISTRY OF METHOD:

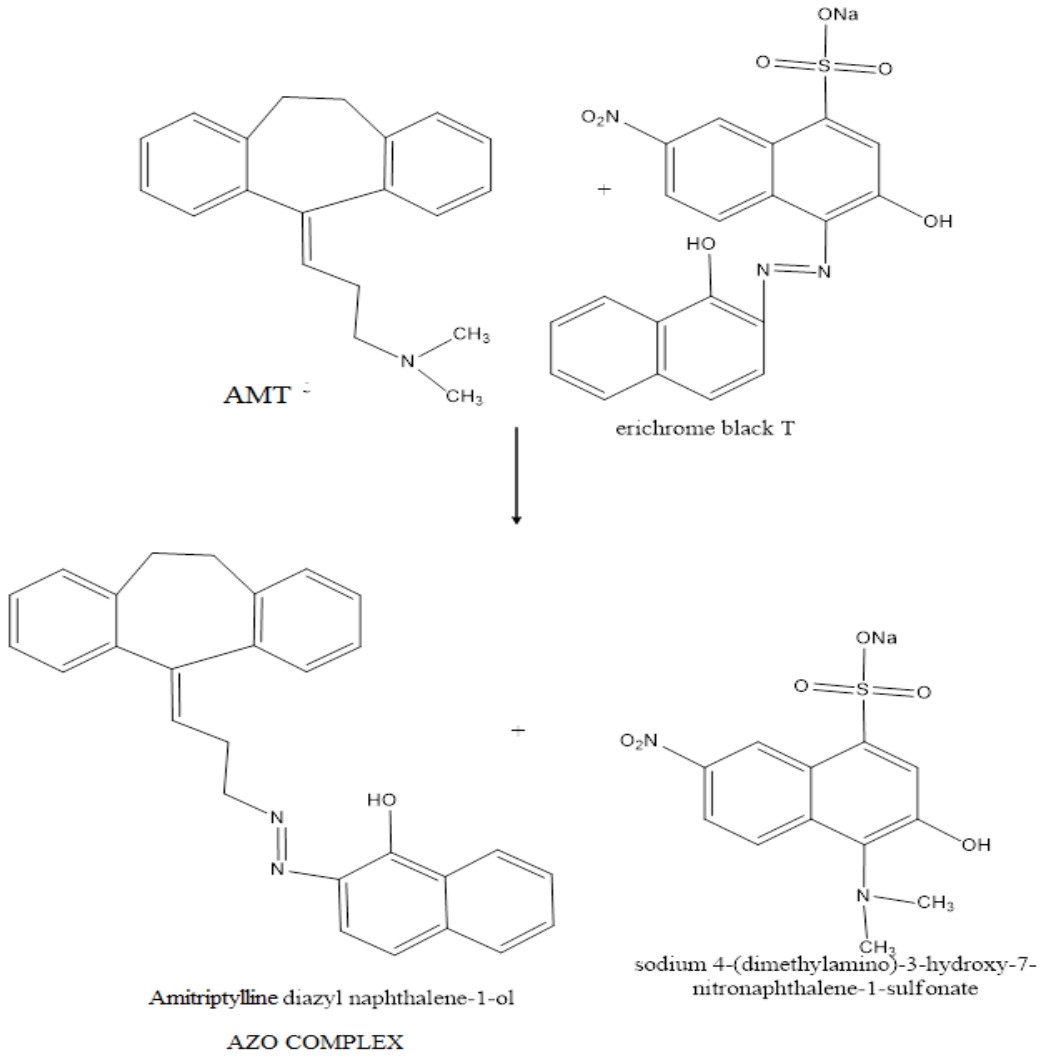

Fig. 1: Chemistry of AMT

The reaction involved in this work is between tertiary amines and suitable coupling reagent which undergoes an electrophilic substitution reaction to produce an azo derivative i.e., Amitryptiline diazyl naphthalene-1-ol and sodium 4-(dimethylamino)-3-hydroxy-7-nitronaphthalene-1-sulfonate. The azo derivatives are colored and consequently have an absorption maximum in the visible region. This reaction forms the basis for AMT determination.

2. STABILITY OF REAGENT:

The absorbance of reagent prepared was stable up to even 8 hours without any fade in colour. The quantitative reaction between drug and the reagent

was observed within few minutes and the absorbance of measured species was stable up to 24 hrs under normal laboratory conditions.

3. EFFECT OF REAGENT CONCENTRATION:

The concentration of EBT indicator depends upon complex formation. Therefore concentration of 0.1% EBT solution is taken. For optimizing the amount of EBT reagent forming complex with drug is selected on trail basis with 0.0, 0.1, 0.5, 1.0, 1.5, 2.0, 4.0mL of AMT (10µg/mL) solution in a 10mL volumetric flask and add 0.5mL and 1.0mL 0.1% EBT reagent separately in each flask and scanned in the range of 400-800nm. The absorbance was linear, as on concentration of drug increases the absorbance was increased. Therefore 1.0mL 0.1%EBT reagent was optimized for the reaction.

4. ABSORPTION SPECTRA:

The EBT reagent has a maximum absorbance at 580nm. The selected species of drug formulation AMT contains an amino group and have maximum absorbance at 201nm which may change with interference of excipients.

The absorbance of colored complex formed on reaction of amino group in AMT and EBT reagent was at range of 570nm. The λ_{max} did not change much but the absorbance was increased and decreased on concentration of EBT reagent taken.

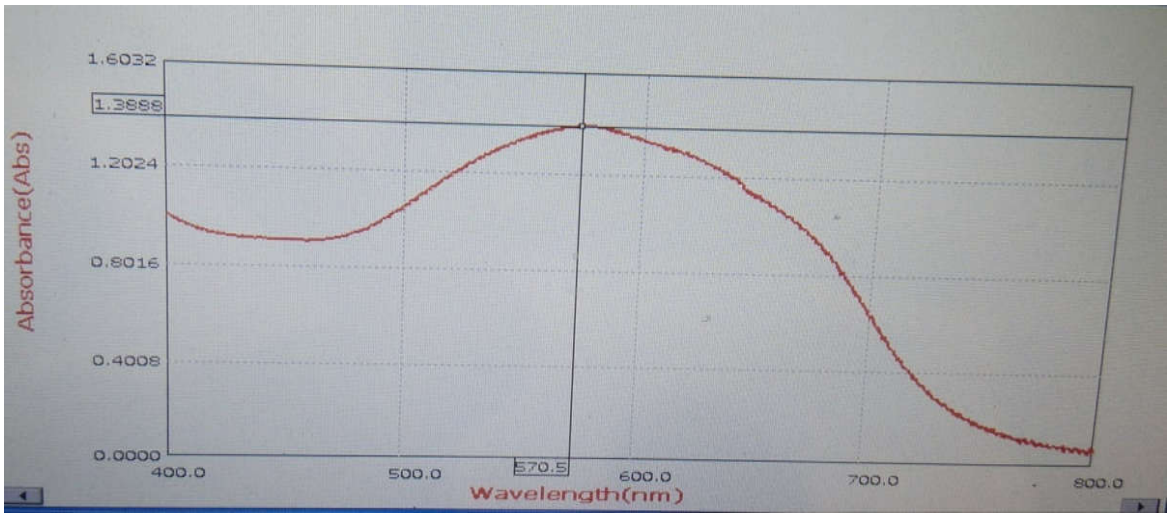

Fig. 2:. Absorption spectra of AMT

5. VALIDATION STUDIES:

The quantitation was achieved with UV detect in at 570nm based on peak areas with linearity concentration ranges 0.1-3.0 µg/ml for amitryptiline hydrochloride with R^2 value 0.998 and calibration curve constructedin fig.2. The %recovery was calculated as 100.02% and %RSD was found to be 0.03. The precision was found to be 0.03 for method precision and 0.029 for intermediate precision. The method is said to be robust at different conditions with no change in optimized method. The LOD's were 0.005µg/ml, LOQ's found to be 0.01µg/ml respectively. All the results are summarized in the following table1.

Table 1: Validation parameters

VALIDATION PARAMETERS	
Linearity	$r^2 = 0.998$
LOD, LOQ	0.005µg/ml,0.01 µg/ml
Accuracy	100.02%
Repeatability	%RSD ≤0.03

6. STABILITY STUDIES:

The stability of drug is determined by exposure in different stress conditions like acid/base stress testing, oxidation, photo and thermal degradation as per ICH guidelines. From our performance we obtained the results of AMT formulation which showed degradation most in base and oxidative degradation, whereas slight degradation in acid and thermal stress conditions and moderate degradation occurs when exposed under normal sunlight and mentioned in table 2. The degradation of excipients is also seen with disturbed base line and irregular peaks at 201nm and formulation drug degradation at 570nm.

Table 2: Stability Studies

STRESS CONDITIONS	ABSORBANCE	% DEGRADATION
Acid degradation	0.9827	As per pharmacopoeia specifications, the official assay limit of the content should NLT 90% and NMT 110% of labeled amount.
Base degradation	1.3497	
Oxidative degradation	1.2864	
Photolytic degradation	0.1583	
Thermal degradation	0.9712	

CONCLUSION:

In present study by using chemical derivatization technique, UV visible spectrophotometric method is developed for determination of anti depressant drug AMT , which is a weekly absorbing analyte containing drug and has tertiary amino group that forms azo derivative on reaction with coupling reagent i.e., EBT indicator which shows stable colored complex among all other reagents. The

developed spectrophotometric method is simple, precise and accurate and stability indicating for amino group in formulation and method is validated as per ICH guidelines. The main advantage of this method is low cost of reagent and apparatus, easily available solvent and instrument used and short analysis time when compared to other reported works.

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