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Full Length Original Research Paper

Development and Validation of a Spectrophotometric method for estimation of Amitriptyline Hydrochloride in Bulk and Tablet Dosage Form

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Abstract: Amitriptyline Hydrochloride (AMI) is a tricyclic antidepressant drug. The aim of this work is to develop a simple, accurate, reproducible and cost effective spectrophotometric method for determination of Amitriptyline Hydrochloride in a pharmaceutical dosage. The absorption maximum of this drug was at 239.0 nm and method has followed linearity in the concentration range 4-24 µg/mL. The generated regression equation was ABS = 0.043 C + 0.035 with R^2 value as 0.9995. The limit of detection (LOD) and limit of quantification (LOQ) were 0.1094µg/ml & 0.3315µg/ml, respectively. Percent relative standard deviation values for the intra-day and inter-day precision were 0.215 and 0.452, respectively, indicated good precision. Results of the recovery studies showed good accuracy of the method. Statistical analysis of results suggests that the developed method is suitable for routine estimation of Amitriptyline Hydrochloride in pharmaceutical formulations.

Keywords: Amitriptyline Hydrochloride, Estimation, Validation, UV spectrophotometery.

INTRODUCTION:

Amitriptyline Hydrochloride (AMI) is a tricyclic antidepressant.[1] It is chemically known as 3-(10,11-Dihydro-5H-dibenzo [a,d] cyclohepten-5-ylidene)-N,Ndimethyl-1-propanamine[2] (Fig.1). This drug is used mainly in the treatment of depression. AMI is official as drug substance as well as Tablet dosage form in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United State Pharmacopoeia (USP). The non aqueous titration method isdescribed in IP[3] and USP[4] andacid-base titration method is described in BP[5] for assay of AMI. Other methods viz. fluorescence polarization immunoassay[6], Ultra-violet (UV) spectrometry[7] have been reported for determination of AMI. Whereas chemometric[8] and chromatographic methods[9-10] have been reported for determination of AMI in combination with drugs. Another literature suggested that determination of AMI withits major metabolites in blood[11] using HPLC. There are only few papers available for estimation of AMI using UV spectrophotometery, but most of these techniques require a sophisticated data processing facility. In this context, we wish to further explore UV spectrophotometery using simple and routinely used spectrophotometer without data processing facility. The present paper describes development and validation of a simple UV spectrophotometric method for assay of AMI in bulk and tablet dosage form.

Materials and methods

Instruments

Shimadzu UV 1800 (Japan) with 1 cm matched quartz cells, connected to computer loaded with UV Prob Software, was employed for this work. Shimadzu AX200 (Japan) digital balance and Spectrolab UCB 40 (Germany) ultrasonicator, were used.

Materials

A gift sample of active pharmaceutical drug (API) of AMI was procured from Astron Research Ltd. (Ahemedabad, Gujarat). Commercially available tablets (Triptomar: containing 10 mg AMI) were obtained from local pharmacy. All the solvents used for this study were of AR grade.

Spectrophotometric method

The standard stock solution of AMI was prepared by transferring, accurately weighed, 100 mg of API to 100 mL of volumetric flask. It was suitably dissolved and volume was made up to the mark by using methanol. This stock solution of the drug was further diluted with the same solvent to obtain 10 µg/mL. The resultant solution was scanned in spectrum mode within 400-200 nm after baseline correction. The spectra of the drug obtained (Fig.2), was used to determine the absorption maxima (λmax). Two different λmax were found, but λmax at 239.0 nm wavelength was selected for this study. Using the stock solution, series of standard working solutions were prepared for AMI at five different levels between 4-24 µg/mL. The absorbances of these solutions were recorded at 239 nm.

Preparation of sample solution

Triptomar tablets (Label claim: 10 mg of Amitriptyline HCl per tablet) were selected for the present work. Twenty tablets were weighed and average weight was calculated. These tablets were crushed and powdered in glass mortar. The tablet powder equivalent to 10 mg of AMI was accurately weighed, transferred to a 100 mL of volumetric flask and diluted up to mark with methanol. This solution was further diluted to obtain 15µg/mL solution with same solvent.

Method Validation:

Linearity and range:

The working standard solutions were prepared by dilution of the stock solution with Methanol in the range of 4-24 µg/ml. The Absorbances of these solutions were measured at 239.0 nm. The relationship between absorption (as a dependant variable) and standard working solution (as an independent variable) were established by simple linear regression method. The regression equation was obtained and this relationship is presented in the calibration curve (Figure 3). The range of solution has been decided according to statistical analysis of regression equation.

Precision:

Intra and inter-day precision studies were performed by measuring the absorbance of standard solution at three different times during the single day and on three subsequent days, respectively. The percentage relative standard deviation (%RSD) was calculated (Table 2).

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Six sets of known concentrations (4-24 µg/ml) were prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the formulae as

$$LOD = 3.3\frac{SD}{S}$$

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$$LOQ = 10\frac{SD}{S}$$

Where, S is value of slopes of calibration plot and SD is calculated using values of y intercepts of regression equations.

Accuracy:

Recovery studies were carried out by applying the developed method to drug sample at which Amitriptyline Hydrochloride corresponding to 80, 100, 120% of label claim was present. Three determinations at each level were performed and results were expressed as % RSD (Table 3).

Results and Discussion:

The generated regression equation was ABS = 0.043 C + 0.035 with R^2 value as 0.9995. Where, ABS is absorbance, C is concentration and R is correlation coefficient. Here, value of R^2 is very close to 1 that suggests that the method is following linearity in the concentration range 4-24 µg/mL of AMI. Results obtained by assay of Amitriptyline Hydrochloride tablet formulation suggest that applicability of developed methods to the tablets, as an average amount found was 100.29% with low % RSD (0.168). Percent relative standard deviation (%RSD) values for the intra-day and inter-day precision were 0.215 and 0.452, respectively, which is under acceptable range. This indicates good precision. LOD and LOQ values were 0.1094 µg/ml & 0.3315 µg/ml, respectively. This suggests that lowest amount of drug that can be detected using this analytical procedure is 0.1094 µg/ml and lowest amount of drug in a sample that can be quantitatively determined with suitable precision and accuracy is 0.3315 µg/ml. Percent (%) recovery was calculated as amount of drug found/drug added X 100. The range of % recovery was 100.11 to 100.45% (with mean 100.20%). Results of the recovery studies indicated good accuracy of the method.

There was no interference from the excipients of tablet formulation. The summary of validation parameters is presented in Table 4.

Conclusion:

A simple, accurate and precise UV Spectrophotometric method for estimation of Amitriptyline Hydrochloride was developed. This method was validated as per ICH guidelines. Results suggest that the developed method is suitable for routine estimation of Amitriptyline Hydrochloride in pharmaceutical formulations.

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Table 1: Results of Assay of Tablet dosage form

Sr. No.	Sample solution concentration (µg/ml)	Amount Found (%)	Mean Amount Found (%)	% RSD*
1	15	100.29	100.36	0.168
2	15	100.35		
3	15	100.59		

*n=3

Table 2: Precision data of Amitriptyline Hydrochloride

Sr. No.	Parameters	Intra-day precision	Inter-day precision
1	Sample solution concentration (µg/ml)	15	15
2	% Assay*	100.41%±0.39	100.59%±0.63
3	% RSD	0.215	0.452

Table 3: Accuracy data of Amitriptyline Hydrochloride

Sr. No.	Accuracy Level	Amount added (µg/ml)	%Recovery *	Mean % Recovery	% RSD
1	I (80%)	12	101.11±0.27	100.29%	0.523
2	II (100%)	15	100.09±0.22		
3	III (120%)	18	100.45±0.31		

*n=3

Table 4: Summary of Validation Parameters

Parameters	Amitriptyline Hydrochloride
λmax (nm)	239.0
Linearity range	4-24 µg/ml
Correlation coefficient (r^2)	0.9995
Slope (m) ± SD*	0.043
Intercept (c) ± SD*	0.035
Regression equation (y = mx+c)	ABS = 0.043 C + 0.035 Simple linear equation
LOD (µg/ml)	0.1094
LOQ (µg/ml)	0.3315
Precision % RSD* Intraday Interday	0.215 0.452
% Recovery	100.29%

*n=6

Fig. 1 Amitriptyline Hydrochloride

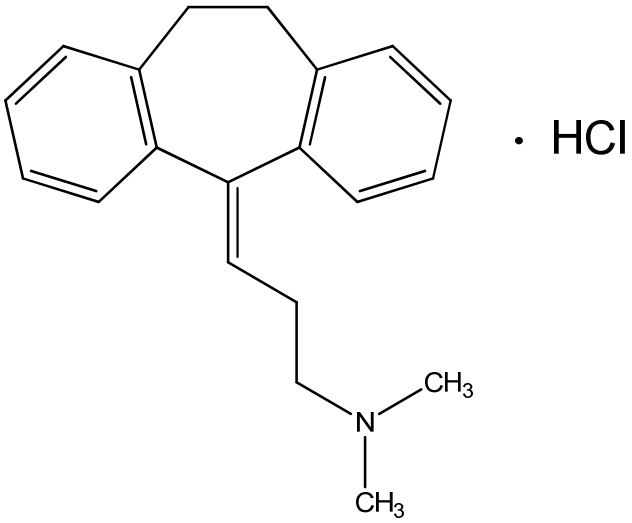


Figure 2: UV spectrum ofAmitriptyline Hydrochloride

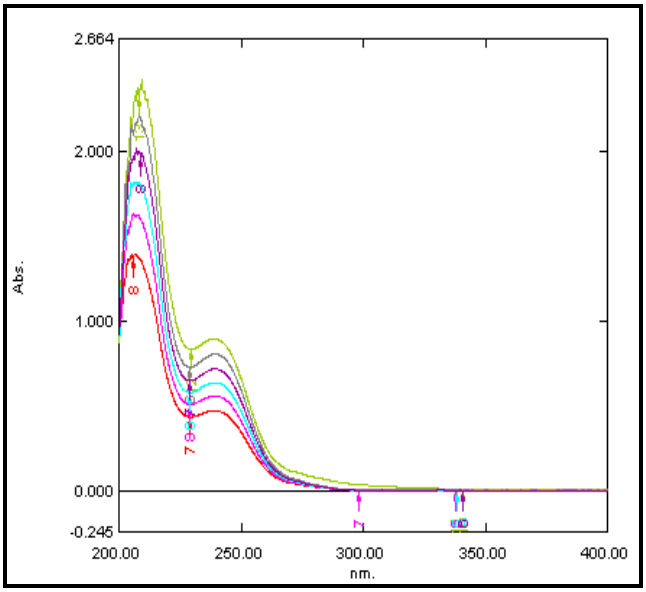
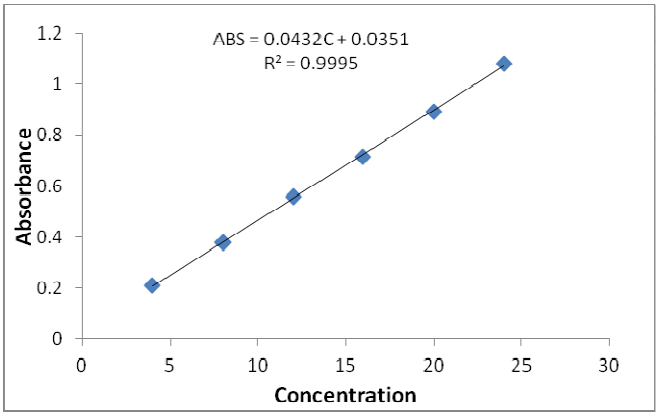


Figure 3: Calibration curve of Amitriptyline Hydrochloride



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