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Der Pharmacia Sinica, 2014, 5(3):9-17

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

UV direct and UV Derivative spectrophotometric methods for the determination of amitriptyline hydrochloride in pure and dosage forms

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ABSTRACT

A simple, sensitive and rapid UV spectrophotometric method and two UV derivative spectrphotometric methods have been developed for the determination of amitriptyline hydrochloride (ATL) in pure and its pharmaceutical formulations. The absorption spectrum of ATL standard solution (10 µg/mL) in methanol was recorded and wavelength of maximum absorbance was found to be 239.0nm. First and second derivative spectra were also recorded for the same solution. From the first derivative spectrum it is found that a valley at 252.4nm showed maximum amplitude and therefore validation of the method was carried out by measuring the amplitudes at this wavelength. Second derivative spectrum has the maximum amplitude in negative valley at 239.0nm hence the second derivative method was validated by measuring amplititudes at 239.0nm. Standard deviation and percent of relative standard deviation were calculated and found to be within the limits. The mean percent of recovery were evaluated at 50%, 100% and 150% concentration levels and found to be within the range 99.4-99.7%. The developed methods were found to be linear within the range of concentrations 5-15µg/mL, and slope, intercept and correlation coefficient were calculated and found to be satisfactory. The developed methods were found to be precise, accurate, robust and stable, therefore readily adapted for routine quality control of ATL by ordinary laboratories. The developed methods were effective for quantitative determination of ATL in bulk and pharmaceutical preparations without any interference of other constitute in tablets of different brand names.

Keywords: Amitriptyline hydrochloride, Derivative spectrphotometry, Amplitude, Pharmaceutical preparations, Assay.

INTRODUCTION

Amitriptyline Hydrochloride (ATL), a dibenzocycloheptadiene derivative is used in depression and nocturnal enuresis. It increases the activity and levels of certain chemicals in the brain. It's chemical name, empirical formula and molecular weight were 3-(10,11-dihydro-5H-dibenzo [a,d] cycloheptene-5-ylidene)-N,N-dimethyl-1-propanamine hydrochloride, $C_{20}H_{23}N \cdot HCl$ and of 313.87 grams per mole respectively. It is supplied as Elavil tablet of 10, 25, 50, 75, 100 and 150mg dosage. Elavil contains not less than 99.0percent and not more than 100.5percent of ATL. The chemical structure of the drug is given in Fig.1.

Several techniques such as UV-Visible spectrophotometry[1-14], UV-Visible and HPTLC[15]' potentiometry, spectrophotometry and atomic absorption spectrometry[16] spectrofluorimetric method[17], gas chromatography[18], capillary electrophoresis[19], voltammetry[20], flow injection potentiometriy [21-22], HPLC and fluorescence[23] were reported for the determination of amitriptyline. Simultaneous estimation of amitriptyline hydrochloride and chlordiazepoxide in tablet dosage forms [24], and amitriptyline and nortriptyline in human breast milk by solid phase extraction [25], amitriptyline hydrochloride and perphenazine by HPLC [26] were also reported. The disadvantages of the most of the visible spectrophotometric methods need to develop color product by treating with a suitable reagent under a set of experimental conditions such as heating or cooling, buffer media and pH,

organic solvents, therefore these methods were time consuming and less sensitive Chromatographic methods are not simple for the routine analysis because they need sophisticated instruments which are not commonly available in the routine laboratories. Therefore it seems necessary to develop a simple and fast identification method for determination of amitriptyline. But UV and UV derivative spectrophotometric methods [27-32] were also comparatively sensitive to other methods, so the author has made some attempts in this direction and succeeded.

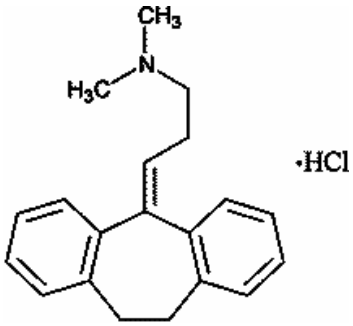

Fig.1: Chemical structure of Amitriptyline HCl

MATERIALS AND METHODS

2.1 Instrumentation

UV-Visible spectrophotometer: An UV-Visible spectrophotometer (UV-3000) with 1cm matched quartz cells was used for the spectral and absorbance measurements.

Digital balance: A Sartorious semi micro digital balance of model number CPA225D was used for weighing purpose.

2.2 Preparation of Standard and Sample solutions

Working standard solution: About 10mg ATL was accurately weighed and transferred into a 100 mL volumetric flask added about 70 mL of methanol and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further 25 mL of ATL stock solution was transferred into a 100mL volumetric flask and diluted up to the mark with methanol, then a series of solutions were prepared by transferring 2.0mL-6.0mL of diluted solution (25µg/mL) into 10mL flasks and diluted

Sample solution: The average weight of five tablets of ATL (Elavil tablets of 100 and 150mg) was accurately calculated and these tablets were grinded well into a uniform powder. Test solution of 100 µg/mL was prepared as explained in preparation of working standard solution by taking an amount of the tablet powder equivalent to10 mg of ATL. Three different concentration solutions at 50%, 100% and 150% of target concentration were also prepared in similar manner from the above stock sample solution.

2.3 Method development

The zero order, first and second derivative spectra were of a solution of 10 µg/mL of ATL were recorded by scanning the absorbance values in the range of wavelength 200-400nm. From the absorption spectrum (Fig.2) it was found that the wavelength of maximum absorbance was 239.0nm. The first derivative spectrum D^1 (Fig.3) crossed the zero point at a wavelength of 239.0nm and producing positive valley at a wavelength of 234.0nm and negative valley at 252.4nm on either side of the zero crossing point of the spectrum. The valley at 252.4nm showed maximum amplitude than the first valley. The second derivative spectrum D^2 (Fig.4) crossed the x-axis at 233.5nm, 251.5nm and 271.3nm leaving negative valley at 239.0nm and a positive valley at 261.5nm. The maximum amplitude was observed at negative valley; therefore the method was validated by measuring amplititudes at 239.0nm

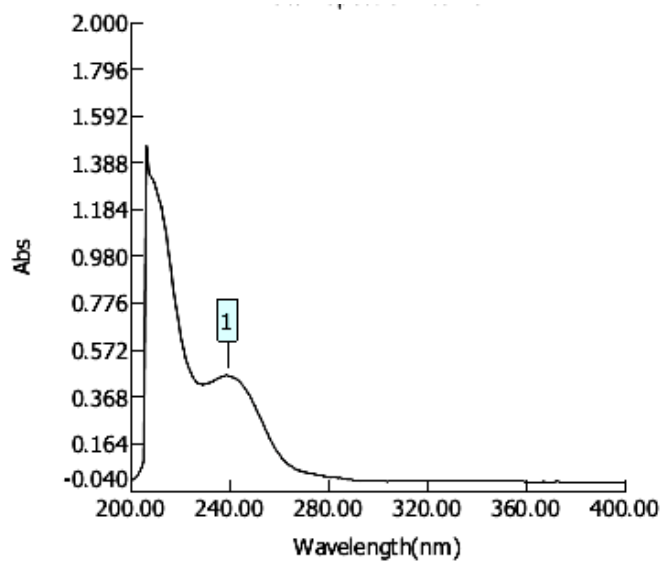

Fig. 2: Absorption spectrum of ATL (10 µg/mL)

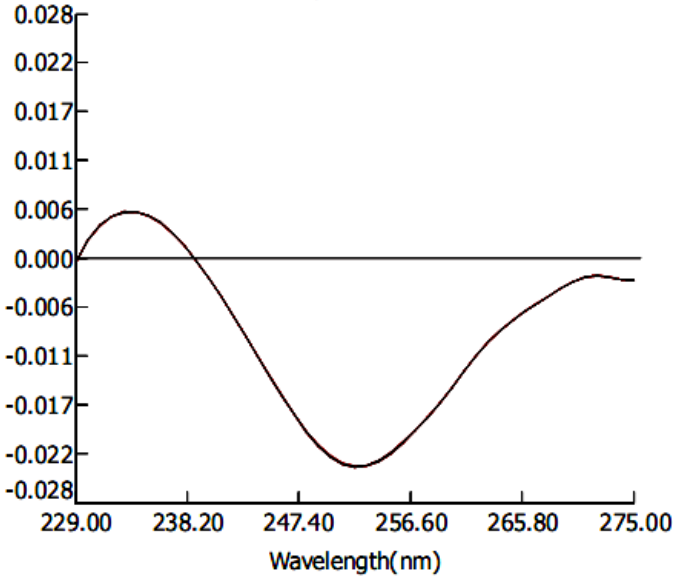

Fig. 3: First derivative spectrum of ATL (10 µg/mL)

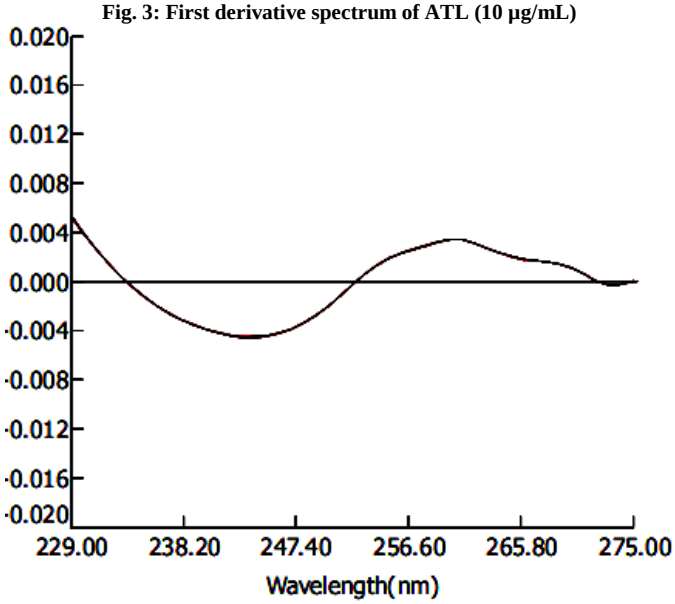

Fig.4: Second Derivative Spectrum of ATL (10 µg/mL)

2.4 Method Validation

Linearity: Different aliquots (2.0mL-6.0mL; 25µg/mL) of working standard solution of ATL were taken in 10mL calibrated tubes, diluted up to the mark with methanol and then kept aside for 10min. The zero order, first order and second order derivative spectra for each of the concentration were recorded over the wavelength range 200-400nm against a reagent blank under similar conditions (Fig.5-Fig.7).

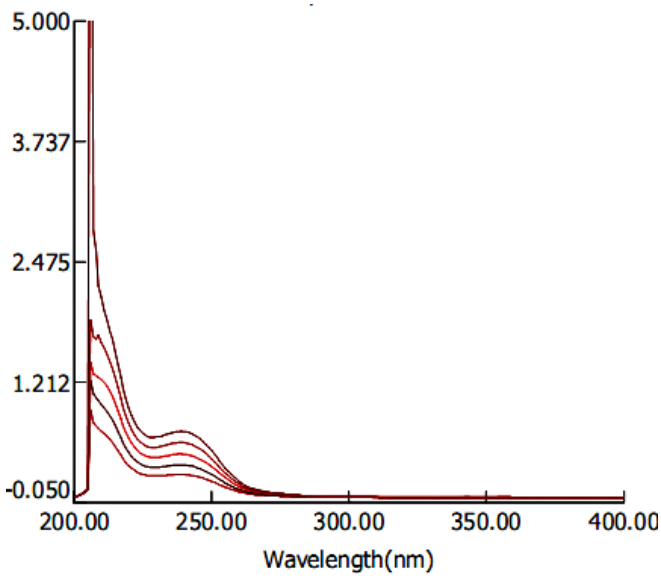

Fig. 5: Absorption spectra of ATL (5.0-15.0 µg/mL)

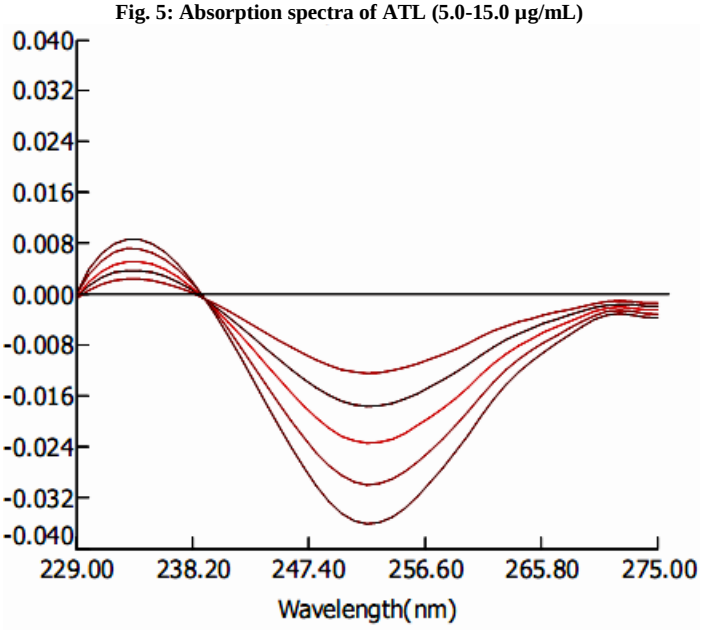

Fig. 6: First Order Derivative spectra of ATL (5.0-15.0 µg/mL)

Calibration plot: In zero order (D^0), a linear straight line was drawn by taking absorbance values on y-axis and concentration on x-axis (Fig.8). In case of derivative method, maximum D^1 and D^2 amplititudes were plotted against concentration of the drug (Fig.9-Fig.10). Linear least squares regression analysis was applied in three cases and slope intercept and correlation coefficient parameters were calculated and were presented in Table-1.

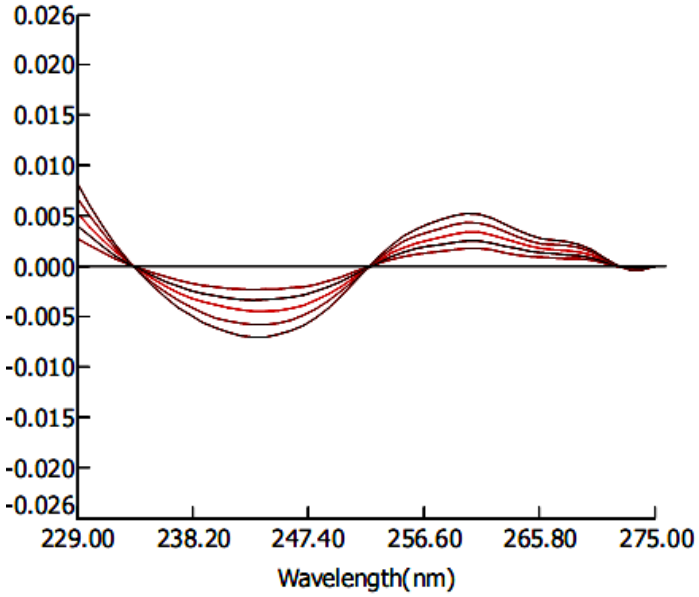

Fig. 7: Second Order Derivative spectra of ATL (5.0-15.0 µg/mL)

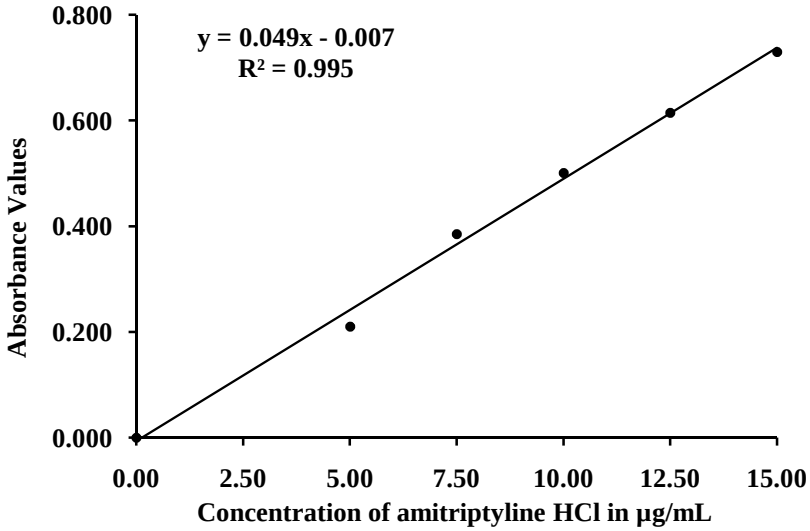

Fig. 8: Linearity plot of absorbance against concentration of ATL

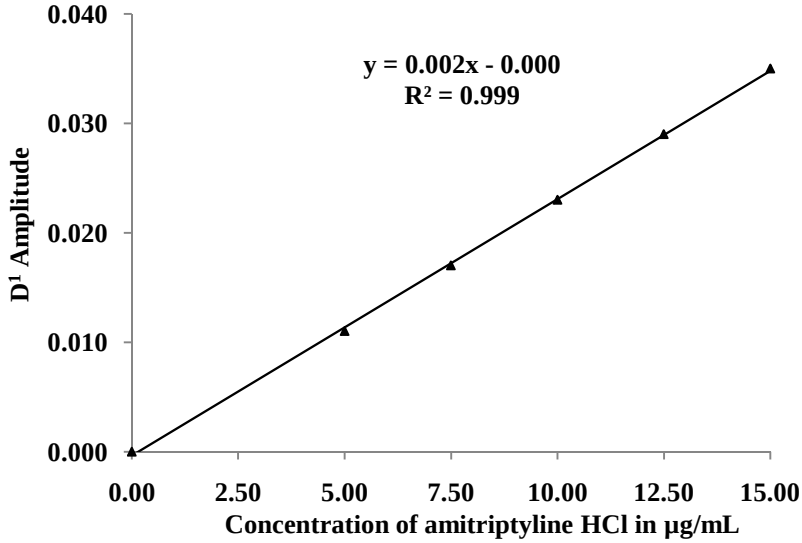

Fig. 9: Calibration plot of first derivative amplitidute against concentration of ATL

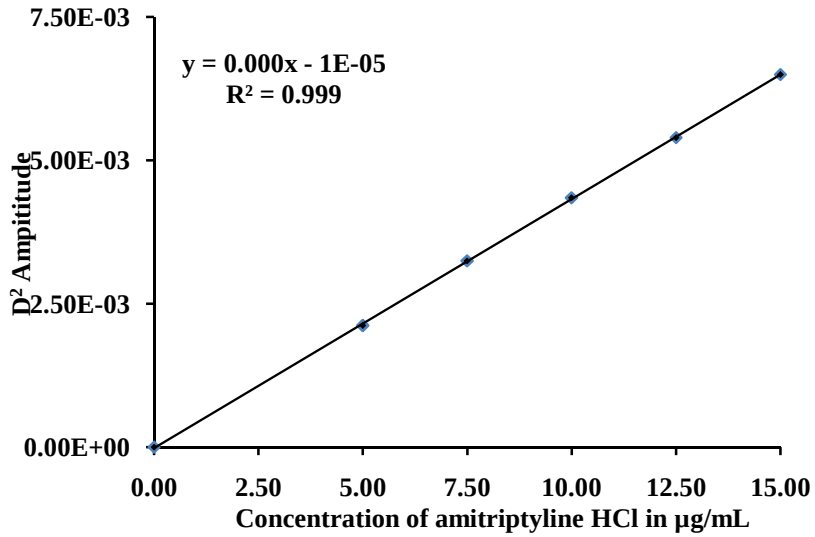

Fig. 10: A linear straight line drawn between second derivative amplitidute and concentration of ATL

Table-1: Linearity of the proposed UV Zero Order Method

S.No.	Concentration µg/mL	Absorbance	D^1Amplitude*	D^2Amplitude*
1	5.0	0.210	0.011	0.00212
2	7.5	0.385	0.017	0.00325
3	10.0	0.501	0.023	0.00435
4	12.5	0.615	0.029	0.00540
5	15.0	0.730	0.035	0.00650
Slope		0.0497	0.0023	0.0004
Intercept		0.0071	0.0004	1.0E.-05
Correlation Coefficient		0.9996	0.9996	0.9999

**D^1 and D^2 are first order and second order derivative spectra*

Precision: Precision (repeatability) of each proposed method was calculated from the absorbance values or maximum amplititudes of five replicates of a fixed amount of ATL in total solution in D^0, D^1 and D^2 respectively. The standard deviation and percent relative standard deviation were calculated for the proposed methods and presented in Table-2.

Table-2: Precision the developed method

S.No	Concentration µg/mL	Zero Order	First Order	Second Order
Average*		0.5068	0.0229	0.0044
Standard Deviation*	10.0	0.0076	0.0002	2.17E-5
%RSD*		1.5117	0.9949	0.4958

** Statistical analysis applied on five replicates of measurements*

Intermediate Precision: To evaluate intermediate precision (reproducibility) measurements were performed on different days under the same experimental conditions. In the present study intermediate precision of each proposed method was ascertained from the absorbance values and amplitudes obtained for five replicates of a fixed amount of ATL in total solution on two different days. The standard deviation and percent relative standard deviation were calculated in each case and presented in Table 3.

Table-3: Study of Intermediate Precision of the proposed method

Statistical parameter	Zero order	First order	Second order
Average*	0.5044	0.0229	0.0044
Standard Deviation*	0.0091	0.0002	1.14E-5
%RSD*	1.7985	0.8392	0.2611

** Statistical analysis applied on five replicates of measurements*

Accuracy: Accuracy, concordance between the measured value and the true or most probable value was determined at three different amounts (50%, 100%, and 150%) of ATL within the Beer's law limits were taken, measurements were made thrice in each concentration. Standard deviation and percent of relative standard deviation were calculated for three replicate measurements at three concentrations. The results were recorded in Table 4(a)-Table 4(c).

Table-4(a) Accuracy of the developed method (Zero derivative)

%Concentration	Amount Added	Amount Found	% Recovery	Mean Recovery
50%	5.0	5.00	100.0%	
100%	10.0	9.91	99.1%	99.7%
150%	15.0	15.00	100.0%	

Table-4(b) Accuracy of the developed method (First derivative)

%Concentration	Amount Added	Amount Found	% Recovery	Mean Recovery
50%	5.0	4.99	99.8%	
100%	10.0	9.98	99.8%	99.4%
150%	15.0	14.80	98.7%	

Table-4(c) Accuracy of the developed method (Second derivative)

%Concentration	Amount Added	Amount Found	% Recovery	Mean Recovery
50%	5.0	4.99	99.8%	
100%	10.0	9.98	99.8%	99.6%
150%	15.0	14.90	99.3%	

Robustness: Robustness of a method is a study of the effect of small variation of the experimental conditions on reproducibility of the measurements. In the present investigation a study of robustness was carried out by making a small change in wavelength (±2) of measurements. The results of robustness of the D^0, D^1 and D^2 spectroscopy were represented in Table 5.

Table-5 Robustness of the proposed method

Wavelength	Absorbance(Zero)	Amplitude(First)	Amplitude(second)
237	0.457	0.04	0.0042
239	0.462	0.05	0.0052
241	0.459	0.05	0.0051

Limit of detection (LOD) and limit of quantization (LOQ): The LOD and LOQ of the proposed method were calculated by using standard deviation of the intercept (σ) and slope (s) of the calibration curve. These were calculated by using the formulae LOD=3σ/s and LOD=10σ/s and are presented in Table 6.

Table-6 LOD and LOQ of ATL

Parameter	Zero Derivative	First Derivative	Second Derivative
LOD	0.2650	0.0261	0.0204
LOQ	0.8850	0.0990	0.0882

2.5 Analysis of formulations

Elavil tablets of 100 and 150mg were analyzed by the validated method by measuring absorbance and amplititude of working standard solution and sample solution. The amount of drug present was evaluated in terms of percent of recovery of six replicates and the results were presented in Table 7.

Table-7 Assay of pharmaceutical formulations

Spectra	Labeled Amount	Amount Found*	SD	%Recovery	% RSD
D^0	100 mg	100.21	0.462	100.21	0.4610
	150mg	149.82	0.716	99.88	0.7169
D^1	100 mg	99.84	1.043	99.84	1.0447
	150mg	150.34	0.945	100.23	0.9429
D^2	100 mg	99.84	1.076	99.84	1.0777
	150mg	150.67	0.587	100.45	0.5844

**Average of six determinations, SD=standard deviation, RSD=relative standard deviation*

RESULTS AND DISCUSSION

The absorption spectrum, first and second derivative spectra of a solution of 10 µg/mL of ATL were presented in Fig.2-Fig.4.The wavelength of maximum absorbance was found to be 239.0nm. The first derivative spectrum (Fig.3) shows the maximum amplitude (valley) at 252.4nm therefore validation of the method was carried out by measuring the amplitudes at this wavelength. In the second derivative spectrum, the maximum amplitude was observed in negative valley at 239.0nm hence the method was validated by measuring amplititudes at 239.0nm. The developed method was found to be linear within the range of concentrations 5-15µg/mL. Slope, intercept and correlation coefficient for the developed method were found to be 0.0497, 0.0071 and 0.9996; 0.0023, 0.0004and 0.9996; and

0.0004, 0.00005 and 0.9999 for zero order, first and second derivative methods respectively. Standard deviation and Percent of relative standard deviation (%RSD) values for zero order, first and second derivative methods were found to be 0.0076&1.5117, 0.00023&0.9949 and 0.00002&0.4958 respectively. The method has been proved robust at ±2nm wavelength variation. The mean percent of recovery and percent of relative standard deviation were evaluated at 50%, 100% and 150% concentration levels. The mean percent of recovery and percent of relative standard deviation were found to be 99.7% (0.35), 99.4% (1.09), 99.6% (0.9). Low % RSD values and high % recovery values support for high accuracy of the methods.

CONCLUSION

The developed UV spectrophotometric methods were effective for quantitative determination of ATL in bulk and pharmaceutical preparations without any interference of other constitute in the formulation. Tablets of different brand names were analyzed and assay of the drug was calculated. The developed methods were found to be simple sensitive, selective, reproducible, and stable. The developed methods could be readily adapted to routine quality control of ATL by ordinary laboratories.

Acknowledgements
The authors are thankful to Acharya Nagarjuna University, Guntur for providing facilities for experimental work and Dr. Reddy's Laboratory for providing gift sample of amitriptyline hydrochloride.

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