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UV SPECTROPHOTOMETRIC AND ITS DERIVATIVE METHODS FOR THE DETERMINATION OF LAMOTRIGINE (LTG) IN PURE AND DOSAGE FORMS

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ABSTRACT

Simple, sensitive and rapid UV Spectrophotometric method and UV derivative Spectrophotometric methods have been developed for the determination of Lamotrigine (LTG) in pure and its pharmaceutical formulations. The absorption spectrum of a solution of 20µg/mL of LTG in methanol was recorded and wavelength of maximum absorbance was found to be 307.0nm. First and second derivative spectra were also recorded for the same. From the first derivative spectrum, it is found that a valley at 335.5nm 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 307.0nm hence the second derivative method was validated by measuring amplititudes at 307.0nm. 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 98.7-100 percent. The developed methods were found to be linear within the range of concentrations 5-30µg/mL. Slope, intercept and correlation coefficient for the developed method were calculated and found to be satisfactory. The methods have been proved robust. The developed derivative Spectrophotometric methods were found to be precise, accurate and stable, therefore readily adapted for routine quality control of LTG by ordinary laboratories. The developed methods were effective for quantitative determination of LTG in bulk and pharmaceutical preparations without any interference of other constitute in tablets of different brand names.

Keywords: Lamotrigine, Derivative spectrophotometer, Amplitude, Pharmaceutical preparations, Assay.

INTRODUCTION

Lamotrigine (LTG) is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorder. It is also used off-label as an adjunct in treating depression. Its chemical name is 3,5-diamino-6-(2,3-dichlorophenyl)-*as*-triazine, its molecular formula and molecular weight are $C_9H_7N_5Cl_2$ and 256.09 grams/mole. LTG is a white to pale cream-colored powder. Lamictal tablets are supplied for oral administration as 25 mg (white), 100 mg (peach), 150 mg (cream), and 200 mg (blue) tablets. Each tablet contains the labeled amount of LTG.. The chemical structure of the drug is given in Fig.1.

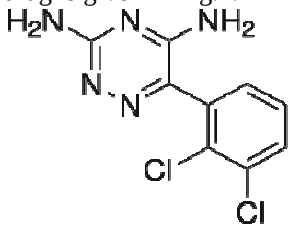

Fig.1: Chemical structure of Lamotrigine (LTG)

Several techniques have been adopted for the determination of Lamotrigine (LTG), a variety of procedures that render the UV determination of LTG have been published. A variety of procedures that render the UV determination of LTG more specific and sensitive, regardless of whether they were determined as single compounds or in mixtures, have been published. Two UV spectrophotometric methods and one spectofluorometric method[1-3] were reported for the determination of LTG in tablets and human plasma. Several visible spectrophotometric methods[4-11] were present for the determination of LTG in pharmaceutical preparations and in biological fluids like urine by charge-transfer complexation, ion-ion association reactions with bromocresol green, bromocresol purple and sulphonthalein dyes. Determination of LTG in plasma was carried out by developing a stability indicating LC method by Pollisetty et.al.[12], simultaneous determination of LTG, zonisamide and carbamazepine in human plasma by high-performance liquid chromatography was reported by Griner-Sosanko et.al.[13] A few HPLC methods were also reported in the literature for the determination of LTG in different biological fluids[14-24]. But only one HPLC method was developed by J. Emami et.al[25] for the determination of LTG and related compounds in tablet formulations. LTG chewable tablets (100 mg) were administered and monitored[26]. Chromatographic techniques are currently used for performing most therapeutic drug monitoring of LTG, but this may change, as automated immunoassays were recently introduced[27]. The developed visible spectrophotometric methods

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involve color developing stage in which drug molecule should quantitatively reacted with reagent to produce stable color which was a time taking process. During color developing, different experimental parameters like temperature (heating or cooling), buffer media (pH), and extracting solvents (organic solvents) were properly maintained to produce stable colored product. In some times the chemical aspects may be complex, to explain the chemistry between drug and reagent, IR spectrum and NMR spectrum in addition to absorption spectrum was essential, therefore visible methods were time taking and lot of care should be taken, where as in UV methods, measuring the absorbance values of drug solution in suitable solvent is sufficient. Derivative methods were more sensitive than direct UV methods or visible methods, but in the literature derivative methods were not reported, hence the author tried in this direction and developed more sensitive derivative Spectrophotometric method for the determination of LTG.

EXPERIMENTAL

Instrumentation: An UV-Visible spectrophotometer (UV-3000) with 1cm matched quartz cells was used for the spectral and absorbance measurements. Semi micro balance (CPA225D) was used for weighing purpose.

Working standard solution: About 10mg of Lamotrizine was accurately weighed and transferred into a 100mL volumetric flask, dissolved in 50mL of methanol and made up to the mark with the same solvent. Further 20 mL of the above solution was transferred in to a100mL volumetric flask and diluted up to the mark. A series of concentration solutions ranging from 10-30µg/mL were prepared by transferring 5.0-15mL of the working standard 20 µg/mL and measurements were made under the optimized conditions.

Method development: After a detailed study of the effects of various parameters on measurements the following procedure was proposed for the assay of LTG by UV & UV derivative spectrophotometry. The absorption spectrum of the working standard solution (20µg/mL) was scanned in the range of wavelength 200-400nm against the reagent blank. The spectrum showed that the wavelength of maximum absorbance was 307.0nm (Fig.2). The proposed method was validated at this wavelength. First and second derivative spectra were recorded for the same concentration. In the first derivative spectrum (Fig.3), it was observed that one positive peak at a wavelength 295.0nm and another negative peak at 335.5nm, and the wave crossed the x-axis at 307.0nm. The negative peak had maximum displacement; hence the proposed method was validated by measuring maximum displacements at 335.5nm. The second derivative spectrum (Fig.4) had two zero crossing at 295.0nm and 335.5nm leaving two positive peaks having peak maximum at 285.0nm and 345.5nm and one negative peak at 307.0nm having maximum amplitude, therefore the developed method was validated at wavelength 307.0nm.

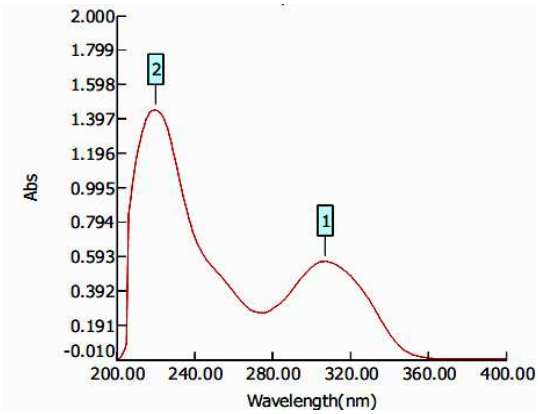

Fig. 2: Absorption spectrum of LTG (20 µg/mL)

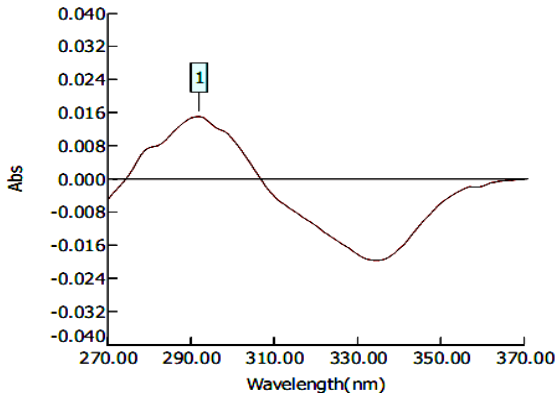

Fig. 3: First derivative spectrum of LTG (20 µg/mL)

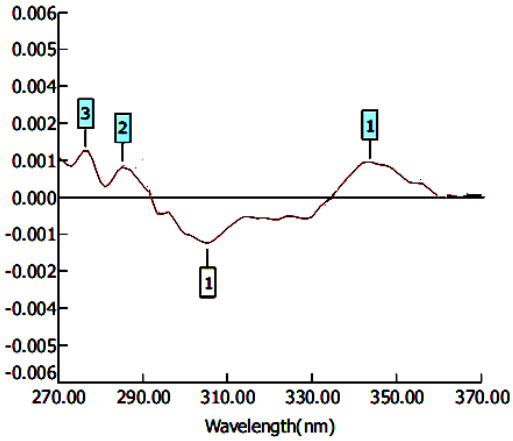

Fig: 4: UV second derivative spectrum of LTG (20µg/mL)

RESULTS AND DISCUSSION

Linearity and Range: To each of 10mL calibrated tubes, different aliquots (5mL -15mL) of standard LTG solution was taken and made up to the mark with methanol and then kept aside for 10min. The zero order, first and second 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). Linearity plots were drawn taking absorbance or amplititudes on x-axis and concentration on y-axis and were shown in Fig.8-Fig.10. The linearity of the data was

evaluated by measuring absorbance and amplitude and the correlation coefficient, slope and intercept were calculated and were presented in Table 2.

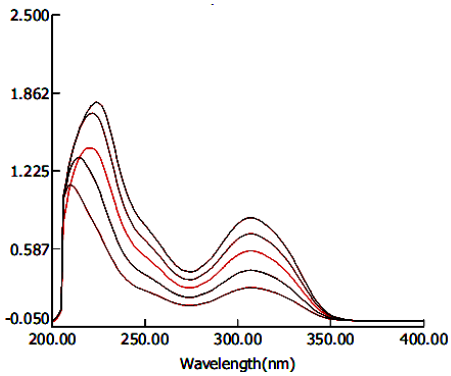

Fig. 5: Absorption spectra of LTG

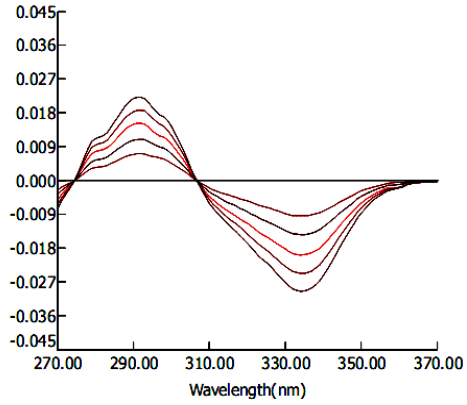

Fig. 6: First Order Derivative spectra of LTG

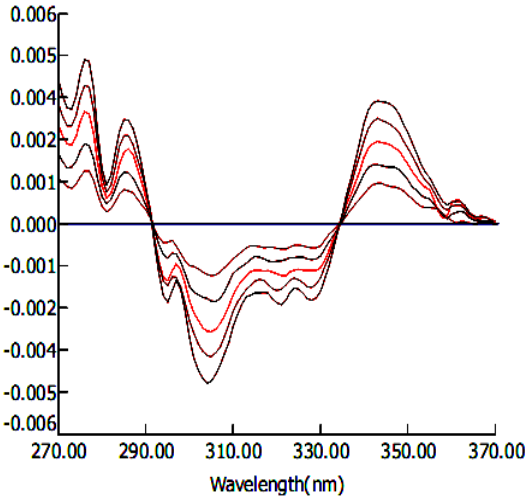

Fig. 7: Second Order Derivative spectra of LTG

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-2.

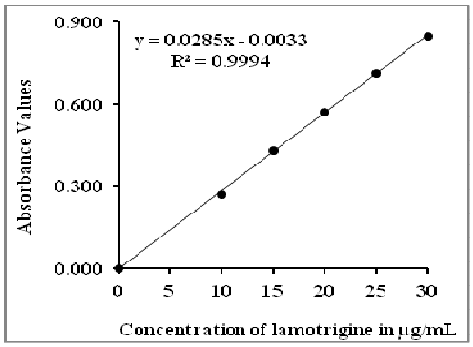

Fig. 8: Linearity plot of absorbance against concentration of LTG

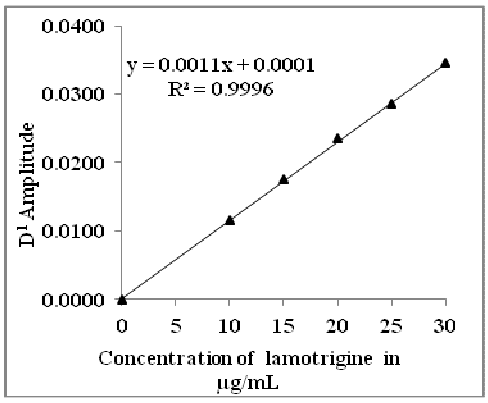

Fig. 9: Calibration plot of first derivative amplitidute against concentration of LTG

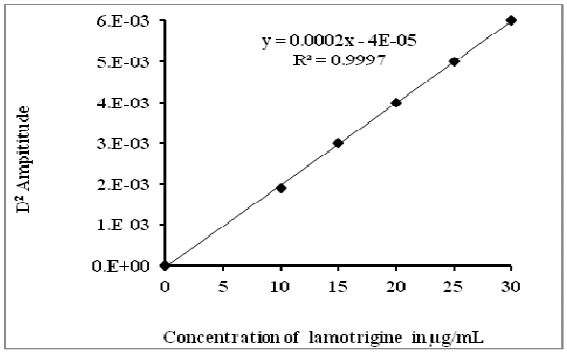

Fig. 10: A linear straight line drawn between second derivative amplitidute and concentration of LTG

Table 1: Statement showing the optimized conditions

Instrument	Wave length	Path Length	Diluent	Concentration Range
UV-Visible Spectrophotometer (UV-3000)	307.0nm	1cm	Methanol	10-30 µg/mL.

Table 2: Linearity of the proposed Method

S. No.	Concentration µg/mL	Absorbance	D^1Amplitude*	D^2Amplitude*
1	10.0	0.269	0.0115	1.90E-3
2	15.0	0.432	0.0175	3.00 E-3
3	20.0	0.571	0.0235	4.00 E-3
4	25.0	0.711	0.0285	5.00E-3
5	30.0	0.849	0.0345	6.00E-3
Slope		0.0287	0.0012	0.0002
Intercept		0.0084	2.0E.-5	4.0E-5
Correlation Coefficient		0.9993	0.9995	0.9997

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 amplitudes of five replicates of a fixed amount of LTG 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-3.

Table 3: Precision for the developed method

S. No	Concentration µg/mL	Zero Order	First Order	Second Order
Average*		0.5704	0.0234	0.0040
Standard Deviation*	20.0	0.0018	1.14E-4	0.0001
%RSD*		0.3185	0.4881	0.9627

* 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 LTG in total solution on two different days. The standard deviation and percent relative standard deviation were calculated in each case and presented in Table-4.

Table 4: Study of Intermediate Precision of the proposed method

Statistical parameter	Zero order	First order	Second order
Average*	0.5690	0.0236	0.0039
Standard Deviation*	0.0016	1.84E-4	2.79E-5
%RSD*	0.2778	0.7816	0.7045

* 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 LTG 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 5(a)-Table 5(c).

Table 5(a) Accuracy of the developed method (Zero derivative)

%Concentration	Amount Added	Amount Found	% Recovery	Mean Recovery
50%	5.0	4.98	99.6%	
100%	10.0	10.0	100.0%	99.4%
150%	15.0	14.8	98.7%	

Table 5(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%	99.7%
150%	15.0	14.9	99.3%	

Table 5(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%	99.7%
150%	15.0	14.9	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-6.

Table 6: Robustness of the proposed method

wavelength	Absorbance(Zero)	Amplitude(First)	Amplitude(second)
305	0.305	0.14	0.0020
307	0.306	0.15	0.0031
309	0.304	0.15	0.0033

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-7.

Table 7: LOD and LOQ of LTG

Parameter	Zero Derivative	First Derivative	Second Derivative
LOD	0.568	0.0617	0.0275
LOQ	0.690	0.0730	0.0380

Assay of pharmaceutical formulations

Sample solution: The proposed UV direct and derivative spectrophotometric method was applied for the analysis of pharmaceutical formulations (Lamictal tablets 200 and 150 mg) of LTG.The average weight of five tablets of LTG was accurately calculated and these tablets were grinded well into a uniform powder. Test solution of 20µg/mL was prepared as explained in preparation of working standard solution by taking an amount of the tablet powder equivalent to10 mg of LTG. Three different concentration solutions at 50%, 100% and 150% of target concentration were also prepared in similar manner. The amount of drug present was evaluated in terms of percent of recovery of six replicates and the results were presented in Table-8.

Table 8: Assay of pharmaceutical formulations

S. No.		Formulation	Labeled Amount	Amount Found*	SD	%Recovery	% RSD
1	D^0	Lamictal tablets	200 mg	199.94	0.871	99.97	0.8713
2		Lamictal tablets	150mg	149.57	1.092	99.71	1.0951
1	D^1	Lamictal tablets	200 mg	200.08	1.376	100.04	1.3754
2		Lamictal tablets	150mg	149.76	1.124	99.84	1.1258
1	D^2	Lamictal tablets	200 mg	199.89	0.472	99.95	0.4723
2		Lamictal tablets	150mg	150.75	0.968	100.50	0.9632

* Average of six determinations, SD=standard deviation, RSD=relative standard deviation, D0, D1andD2 were represent zero, first and second order derivatives

CONCLUSION

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

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