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ANALYTICAL METHOD DEVELOPMENT AND ITS VALIDATION FOR ESTIMATION OF LAMOTRIGINE IN BULK AND TABLET BY UV- SPECTROSCOPIC METHOD

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ABSTRACT: Three simple precise and economical UV spectrophotometric methods have been developed for the estimation of Lamotrigine in bulk and pharmaceutical formulation. Chemically, Lamotrigine is 3,5-diamino-6-(2,3-dichlorophenyl). It is used as anticonvulsant. Lamotrigine has absorbance maxima at 305 nm in zero order spectrum method (Method A). In first order derivative spectra, showed sharp peak at 291 nm when n=1 (Method B). Method C has based on calculation of area under curve (AUC) for the analysis of Lamotrigine in the wavelength range of 281.0 nm -310.0 nm. The drug followed Beer-Lambert's law in the concentration range of 5-50µg/ml. The result of analysis was validated statistically and by recovery studies and were found to be satisfactory.

Keywords: Lamotrigine, UV-Visible Spectrophotometry, zero order spectrum, first order spectrum, Area under curve (AUC)

INTRODUCTION:

Lamotrigine is an anticonvulsant agent[1,2]. Chemically it is 3,5-diamino-6-(2,3-dichlorophenyl)[1-4]. In vitro pharmacological studies suggest that Lamotrigine inhibits voltage-sensitive sodium channels, thereby stabilizing neuronal membranes and consequently modulating presynaptic transmitter release of excitatory amino acids (e.g., glutamate and aspartate)[4-8]. It is also listed in Merck index[1], Martindale[2], USP.[3] and I. P.[4] Literature survey reveals that HPLC[9-16] and HPTLC[17-18] methods for determination of Lamotrigine are reported. Hence the objective of the work is to develop new UV Spectrophotometric methods for estimation of Lamotrigine in bulk and tablet dosage form with good accuracy, simplicity, precision and economy.

MATERIALS AND METHODS

Instrument

A Shimadzu UV/VIS Spectrophotometer 1700 was used with 1cm matched quartz cells and spectral bandwidth of 2 cm.

Materials : Standard gift sample of Lamotrigine was procured from Lupin Pharma. Ltd., Pune. Tablets of Lamotrigine were procured from marketed commercial brand.

Stock Solution: : Accurately about 10 mg of Lamotrigine was weighed and transferred to 100 ml volumetric flask. 20 ml of distilled water was added to dissolve the drug completely with vigorous shaking. Then the volume was made up to the mark with the distilled water to give stock solution of concentration 100 µg/ml.

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Method A

Aliquots of standard stock solution were pipetted out suitably diluted with distilled water to get final concentration of 5, 10, 15, 20..... to 50 µg/ml. of standard solutions. The solutions were scanned in the spectrum mode from 400 nm to 200 nm wavelength range and the zero order derivative spectra were obtained (fig.1). The maximum absorbance of Lamotrigine was observed at 305.0 nm. The drug followed the Beer-Lambert's law in the concentration range of 5-50 µg/ml. The calibration curve was plotted as absorbance against concentration of Lamotrigine. The coefficient of correlation(r), slope and intercept values of this method are given in Table 1. The concentrations of sample solutions were determined from calibration curve.

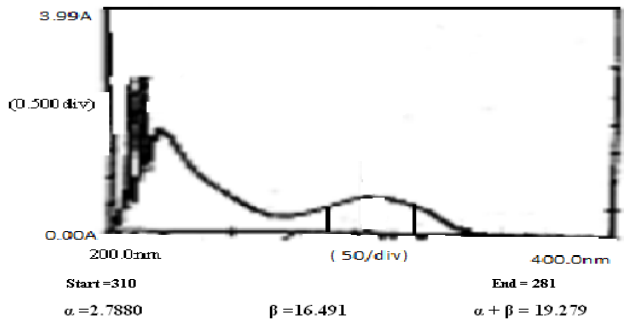

Fig.1: Zero order spectrum of Lamotrigine

Method B

The first order derivative spectra at n=1 showed a sharp peak at 291.0 nm (fig. 2). The absorbance difference at n=1 (dA/dλ) was calculated by the inbuilt software of the instrument which was directly proportional to the concentration of the standard solution. The standard solutions were scanned in the first order derivative spectra. A calibration curve was plotted taking the absorbance difference ((dA/dλ) against the concentration of Lamotrigine. The coefficient of correlation (r), slope and intercept values of this method are given in table 1. The method was applied for determination of concentration of sample solution.

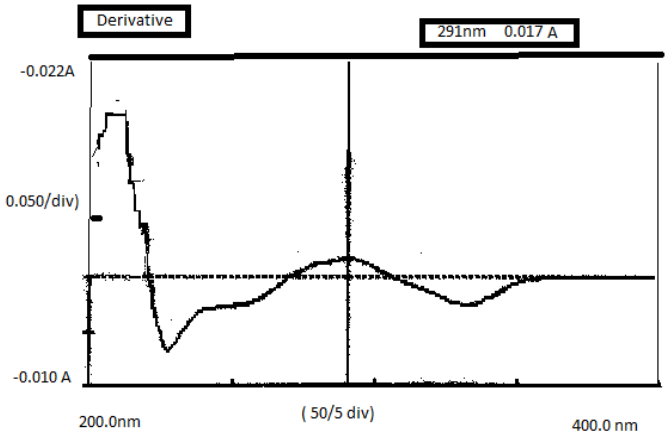

Fig.2: First order derivative spectrum of Lamotrigine

Table 1: Optical Calibration Curve of Lamotrigine

Parameters	Method A	Method B	Method C
max (nm)/wavelength range(nm)	305.0 nm	291.0 nm	281.0-310.0 nm
Beer's – Lambert's range(µg/ml)	5-50 µg/ml	5-50 µg/ml	5-50 µg/ml
Coefficient of correlation (r^2)	0.9981	0.9963	0.9988
Regression equation : Y = mx + c			
a – Slope (m)	0.03425	0.0065	0.251
b – Intercept (c)	0.000	0.000	0.000
LOD (µg/ml)	0.08855	1.5738	0.0174
LOQ (µg/ml)	0.26838	4.769	0.0529
Molar absorptivity	2.92×10^{-3}	6×10^{-5}	-

Method C

The AUC (Area under curve) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. This wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. Suitable dilutions of standard stock solution (100 µg/ml) of Lamotrigine were prepared and scanned in the spectrum mode from the wavelength range 400 nm to 200 nm (Fig. 3). The wavelength range for Lamotrigine was selected from 281.0 nm to 310.0 nm and measured AUC for each dilutions of Lamotrigine. The calibration curve was plotted as AUC against concentration of Lamotrigine. The method was checked by analyzing the samples with known concentration. As the results obtained were satisfactory low, the method was applied for pharmaceutical formulations.

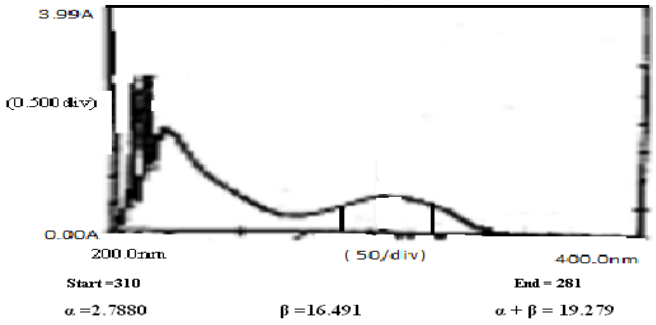

Fig 3: Area under curve of Lamotrigine

Analysis of tablet formulation

For estimation of Lamotrigine in tablet formulation by all the methods, twenty tablets were weighed and triturated to the fine powder. Tablet powder equivalent to 10 mg of Lamotrigine was weighed and dissolved in 20 ml distilled water. It was kept for ultrasonication for 45 min. Finally, the volume was made up to the mark with distilled water.; it was filtered through Whatman filter paper no. 41 to get tablet stock solution of concentration 100 µg/ml. Various dilutions of tablet stock solution were prepared and analyzed for six times by all three methods and concentrations of Lamotrigine in tablet formulation T1 were calculated by all three methods (Table II). All these methods were validated according to ICH guidelines. Recovery studies were carried out at three different levels i.e. 80 %, 100 % and 120 % by adding the pure drug (8 mg, 10 mg and 12 mg respectively) to previously analyzed tablet powder sample (10 mg) as per ICH guidelines and percentage recovery was calculated as shown in Table III. All the methods A, B and C were validated for linearity, accuracy and specificity.

Table 2: Estimation of Lamotrigine formulation tablet

Method	Tablet Formulation	Label claim	Amount found	% mean	S.D	C.O.V	S.E
A	T1	25	24.09	100.036	0.6656	0.6654	0.2977
B	T1	25	25.09	100.36	0.9642	0.9638	0.3936
C	T1	25	24.96	99.69	0.246	0.244	0.096

Table 3: Recovery Study Data

Method	Level Of % Recovery	Amount present (mg/tab)	Amount of std drug added	Total amount recovered (mg)*	% Recovery*	S.D	C.O.V	S.E
A	80	10	8	17.82	99.00	0.4300	0.4328	0.2483
	100	10	10	19.94	99.70	0.2754	0.2770	0.1590
	120	10	12	21.88	99.45	0.1375	0.1383	0.0793
B	80	10	8	17.94	99.89	0.2761	0.2764	0.1594
	100	10	10	20.16	100.2	0.5074	0.5063	0.2930
	120	10	12	22.10	100.6	0.6846	0.6805	0.3953
C	80	10	8	17.80	99.27	0.348	0.350	0.200
	100	10	10	19.94	99.41	0.256	0.258	0.147
	120	10	12	21.79	99.34	0.267	0.269	0.154

RESULT AND DISCUSSION

All methods A, B and C for the estimation of Lamotrigine in tablet dosage form were found to be simple, accurate, precise, specific and reproducible. Beer-Lambert's law was obeyed in the concentration range of 5-50 µg/ml. The values of standard deviation were satisfactory low and the recovery studies were close to 100 %. Lamotrigine showed a broad spectrum, the derivative spectroscopy method applied has the advantage that it locates the hidden peaks in the normal spectrum when the spectrum is not sharp and it also eliminates the interference caused by the excipients present in the formulation. The AUC method has advantage that it is applicable to be drug which shows the broad spectra without a sharp peak. Hence these methods can be useful in the routine

analysis of Lamotrigine in bulk drug and formulations.

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