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DEVELOPMENT AND VALIDATION OF UV VISIBLE SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF LAMOTRIGINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Lamotrigine is an anticonvulsant drugs used in the treatment of epilepsy and bipolar disorder A simple, sensitive, accurate and reproducible UV/visible spectrophotometric method was developed for the determination of lamotrigine in bulk and pharmaceutical dosage forms. The solvent distilled water and wavelength corresponding to maximum absorbance for the drug was found at 300nm. Drug obeyed beer's law in concentration range of 1to 5 ug/ml. With a correlation coefficient of 0.9990. The linear regression equation obtained was y =0.1371x+0.0168, where y is the absorbance and x is concentration of pure drug solution. The method was validated for several parameters such as Linearly, Accuracy, precision, Robustness as per ICH guidelines. The % recovery value with is close to 100% indicates reproducibility of the method and absence of interference of the excipients present in the formulation. The authors conclude that the proposed spectrophotometric method for the estimation of lamotrigine can be used for routine analysis of lamotrigine is bulk as well as in tablet dosage form.

KEYWORDS: Lamotrigine, Dimethyl sulfoxide, Spectrophotometry.

INTRODUCTION

The aim of the present investigation was to devise a straightforward, exact, and reliable Spectrophotometric assay technique, and to validate it for the quantification of Lamotrigine

in solid pharmaceutical dosage forms. Dimethyl sulfoxide was used to make the standard stock solution. It was discovered that lamotrigine's λ max was 300 nm. Specificity, linearity, accuracy, precision, robustness, and solution stability of the approach were all validated. With a correlation coefficient of 0.9990, the approach demonstrated linearity within the drug concentration range of 1 to 5 µg/ml. When it came to repeatability, the precision (or relative standard deviation, or RSD) among the six-sample preparation was 0.98%, and the intermediate precision (or RSD) was 0.79%. The recovery (accuracy) ranged from 98.52% to 100.61%.[1]

Lamotrigine is a type of medication used to treat bipolar disorder and epilepsy. Lamotrigine (figure 1), a phenyl triazine that differs chemically from other anticonvulsants, seems to prevent excitatory neurotransmitter release from neurons via voltage-sensitive sodium channels and voltage-gated calcium channels.[2]

Chemically, lamotrigine is 6-(2, 3-Dichlorophenyl)-1, 2, 4-triazine-3, 5-diamine. Its molecular weight is 256.09 gm/mole and its formula is C9H7N5Cl2. Lamotrigine, a phenyl triazine class antiepileptic drug (AED) that has been effectively utilized to treat essential trigeminal neuralgia, is structurally unrelated to other antiepileptic medications now on the market.[3]

Unlike other anticonvulsants chemically (since lamotrigine is a phenyl triazine). When used as a monotherapy, lamotrigine has comparatively little side effects and doesn't require blood monitoring. Moreover, lamotrigine stabilizes mood. For epilepsy, a starting dose of less than 1 mg is advised. Typically, the epilepsy therapeutic spectrum is 300–500 mg daily.[4] The usual practice for lamotrigine dosage adjustments is to make moderate increases and decreases. Compared to most other psychiatric drugs, extremely minor dosage variations frequently have dramatically different effects; as little as 10% more or less may make a visible impact. A therapeutic response may need weeks or months of consecutive dose escalations.[5]

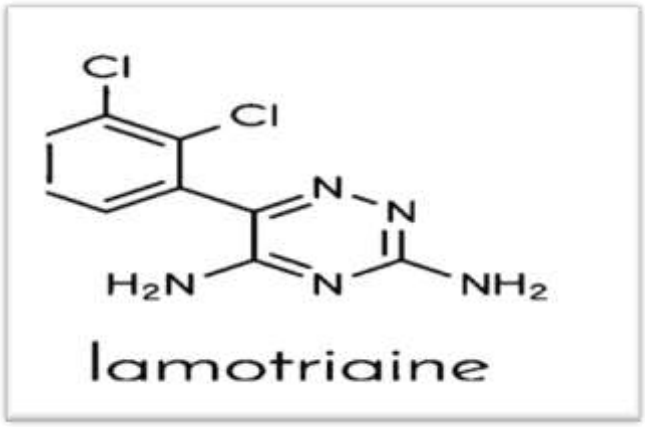

Fig-1 6-(2, 3-Dichlorophenyl)-1, 2, 4-triazine-3, 5-diamine.

Lamotrigine from pharmaceutical formulation and HPLC methods also applied for the determination of Lamotrigine. So far to our present knowledge, no such validated stability indicating spectrophotometric assay method for the determination of Lamotrigine in pharmaceutical formulation was available in literature. Moreover, spectrophotometric method can be the first choice of chromatographers among the High-performance liquid chromatography; Reflectance Near-Infrared and Gas Chromatography methods. So, development is based on spectrophotometric method. This paper deals with the validation of the developed method for the assay of Lamotrigine from its dosage form (tablets).[6]

MECHANISM OF ACTION

- Mechanism of action is similar to that of phenytoin and carbamazepine.
- It brings to inactive sodium channels, suppress the release of excitatory amino acid glutamate and inhibit sodium current. Reduction in neuronal Na+ concentration decrease post – tetanic potentiation [PTP] which is responsible for spread of seizure discharge in the brain and shortens the duration of after discharge.[7]
- It also enhances action of gamma amino – butyric acid, an inhibitory neurotransmitter. it results in reduction of pain – related transmission of signals along nerve fibers.

Determination of λ max

By appropriate dilution of standard stock solutions of Lamotrigine in dimethyl sulfoxide containing 2µg/ml of Lamotrigine, dilutions were made and scanned on uv-visible double beam spectrophotometer in the range of 200- 800 nm against distilled water as blank. Wavelength of maximum absorption was determined for drug. Lamotrigine showed maximum absorbance at 300nm.[8]

MATERIALS AND METHODS

Experimental Materials

Boisar, India's Aarti Drugs Ltd. supplied the lamotrigine standard. The market provided the lamotrigine tablets with 25 mg of lamotrigine and the inactive component employed in the medication matrix. Spectrochem Pvt. Ltd., Mumbai (India) provided water and dimethyl sulfoxide of analytical quality.[9]

Instrumentation

UV-Visible double beam spectrophotometer PharmaSpec 1700 (Shimadzu, Kyoto, Japan) with matching quartz cells (1cm) was the spectrophotometer equipment used for the development and validation of this assay method.[10]

Diluent Preparation

Dimethyl sulfoxide is employed as a diluent**.**

Standard preparation

Dimethyl sulfoxide was used as a diluent to dilute the 25.00 mg of lamotrigine to volume in a 50 ml volumetric flask, resulting in a lamotrigine standard stock solution with a concentration of 25 mg/ml.

In a 10-milliliter volumetric flask, add an additional 1 millilitre of this stock solution, and dilute to the mark.

Next, use diluents to make up to the mark in a 10-milliliter volumetric flask containing 1 millilitre of this stock solution.

Test preparation

Weighing ten tablets allowed us to calculate their average weight. A volumetric flask was filled with the average amount of the weighted tablet from these. Once the diluents were added, around 25 ml, they were sonicated for at least 30 minutes while being shaken occasionally. Following a return to room temperature, the content was volume-diluted using dimethyl sulfoxide. Using Watt's man filter paper, the material was filtered. The level of concentration attained. In a 25 ml volumetric flask, take 1 ml of this filtrate solution and dilute it to the mark.[11]

METHOD VALIDATION

Specificity study

The method's specificity was evaluated in relation to location. The alleged placebo's excipients, which were obtained from the placebo solution, interfered when they were added to a medicinal dosage form.[12]

Linearity

The capacity of an analytical method to produce test findings that are exactly proportionate to the concentration (amount) of analyte in the sample, within a specified range, is known as linearity.

The absorbance of each standard solution was plotted versus concentration at 285 nm to determine the linearity of the suggested approach. The absorbance values are listed in the table. It was observed that the naproxen exhibited linearity at 1-200 gg/ml of concentration. It was the regression coefficient. determined to be 0.991.[13]

Preparation of drug solutions for linearity

Precisely measure and transfer 10 mg of the working standard for Lamotrigine into a 10 ml clean DIY volumetric flask. Next, add around 7 ml of diluents and sonicate to fully dissolve the material. Adjust the volume using the same solvent.[14] (Stock remedy)

Preparation of Level – 1 (1mg/ml of Lamotrigine): Pipette out 1ml of stock solution in to a 10ml volumetric flask and make up to mark by using diluent.

Preparation of Level – 2 (2mg/ml of Lamotrigine): Pipette out 2ml of stock solution in to a 10ml volumetric flask and make up to mark by using diluent.

Preparation of Level – 3 (3mg/ml of Lamotrigine): Pipette out 3ml of stock solution in to a 10ml volumetric flask and make up to mark by using diluent.

Preparation of Level – 4 (4mg/ml of Lamotrigine): Pipette out 4ml of stock solution in to a 10ml volumetric flask and make up to mark by using diluent.

Preparation of Level – 5 (5mg/ml of Lamotrigine): Pipette out 5ml of stock solution in to a 10ml volumetric flask and make up to mark by using diluent.

Precision

Repeatability and intermediate precision were taken into account when determining precision. In terms of relative standard deviation (RSD), precision (repeatability and intermediate precision) was expressed.

The precision under the same operating conditions over a brief period of time is expressed by repeatability. Intra-day precision is another name for repeatability. %RSD was computed after six drug solutions. The outcomes are displayed in the table.

Variations that occur within laboratories—different days, different analyzers, different equipment, etc.—are expressed by intermediate precision. By making six solutions, each containing 1 ug /ml, it was assessed and the percentage RSD was computed. The outcomes are displayed in the table.

It was discovered that the percentage RSD of intermediate precision and repeatability was less than 2.0%.

The intra-assay test determines if a procedure yields reliable findings for a particular batch. The evaluation involved the assaying of three standard preparations at three distinct concentrations (40 ug/ml, 80 ug/mg, and 160 ug/ml), and the percentage RSD was computed. Results are displayed in the table.[15]

Accuracy

The degree to which the value found and the value acknowledged as a conventional true value or an established reference value agree is expressed as the accuracy of an analytical technique. Sometimes, this is referred to as trueness. Recovery verified that the suggested procedure was accurate.[16]

Recovery studies were used to verify the accuracy of the suggested approach by adding standard drug solution to a sample of pre-analysed tablets at three distinct concentration levels within the linearity range. According to ICH recommendations, recovery tests were conducted at 50, 100, and 150 percent of the standard concentration to verify the correctness of the suggested approach. Standard concentrations at three separate levels within the linearity range (2 ug/ml, 4 ug/ml, and 6 ug/ml) were added to a test solution containing 1 ug/ml. The correctness of these spiking solutions was verified. % The recovery was computed using the subsequent formula.[17]

$$\% \text{ Recovery} = \frac{\text{spiked concentration} - \text{test concentration}}{\text{Standard concentration}}$$

A table presented the recovery study results.

Robustness and Ruggedness

An analytical procedure's robustness is a measure of how well it can withstand slight, intentional changes in method parameters and shows how reliable it is under typical use.

The analytical method's resilience and ruggedness were determined through the analysis of an identical sample under a range of standard test settings, such as wavelength variations and different analysts. 50 ug/ml of the drug (for a wavelength change) and 60 ug/ml of the drug (for various analysts) were manufactured. Six calculations of the above concentration are made using the aforementioned parameter, and the resulting solution's absorbance was tested. A table with the findings was provided.[18]

RESULTS AND DISCUSSION

The λ maxima of Lamotrigine in standard and test preparation was found to be 300 nm from its spectrum (Fig. 1 & 2)

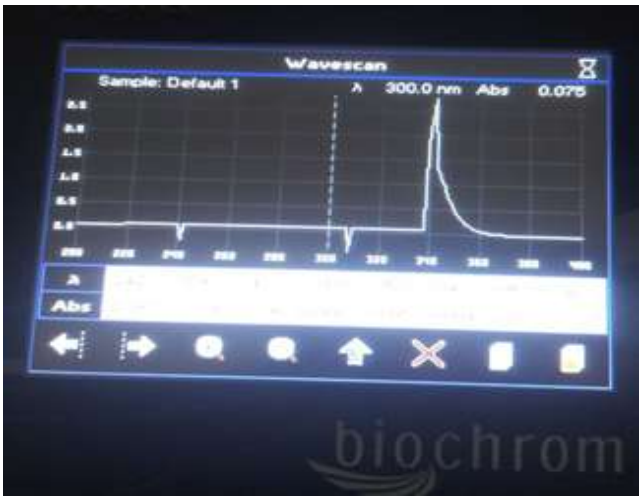

Fig. 1: UV spectrum of Lamotrigine in Standard solution.

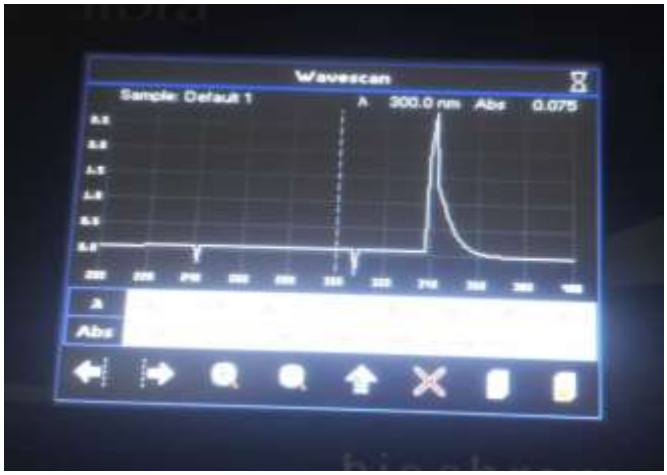

Fig. 2: UV spectrum of Lamotrigine in Sample solution.

Lamotrigine showed linear absorption from 1- 5µg/mL. The correlation coefficient (r) was found to be 0.998.(Fig. c) The stability of solutions of formulation was determined by measuring the absorbance at 300 nm at periodic intervals.

Table: 1 Data Of Calibration Curve.

Concentration (*ug/ml*)	Absorbance
1 ug/ml	0.156
2 ug/ml	0.294
3 ug/ml	0.431
4 ug/ml	0.573
5 ug/ml	0.718

Y =0.1371x+0.016 **R^2 = 0.9987** **Slope = 0.137**

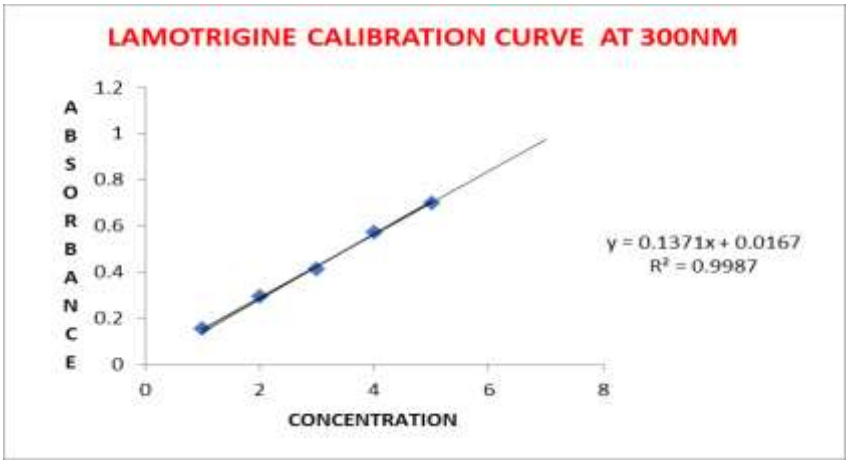

Fig 3: Lamotrigine Calibration Curve.

Table-2: Evaluation data of precision study.

SET	INTRADAY(n=6)	INTERDAY(n=6)
1	0.253	0.252
2	0.256	0.255
3	0.252	0.250
4	0.258	0.257
5	0.255	0.256
6	0.250	0.256
Mean	0.25400	0.25433
Standard deviation	0.00289	0.00274
%RSD	1.142	1.074

Table-3: Evaluation data of Accuracy study.

Concentration ug/ml	Test	standard	spiked	% Recovery
50% 2ug/ml+1ug/ml	0.22	0.250	0468	99.2%
	0.19	0.255	0.445	100%
	0.2	0.250	0.452	100.8%
			AVERAGE	100.2
			SD	0.8
			%RSD	0.86
100% 4ug/ml+1ug/ml	0.13	0.570	0.71	100%
	0.09	0.572	0.66	99.45%

	0.14	0.573	0.71	99.48%
			AVERAGE	99.65
			SD	0.302
			%RSD	0.304
150% 6ug/ml+1ug/ml	*0.11*	*0.643*	*0.75*	*99.54%*
	0.12	*0.645*	*0.76*	*99.23%*
	0.11	*0.642*	*0.72*	*101.2%*
			AVERAGE	99.98
			Standard deviation	1.06
			%RSD	1.063

Table 4: Evaluation data of Repeatability study.

Concentration ug/ml	Absorbance
2ug/ml	0.250
2ug/ml	0.255
2ug/ml	0.250
2ug/ml	0.257
2ug/ml	0.256
2ug/ml	0.248
Mean	0.252
SD	0.00476
%RSD	1.89

Table 5: Evaluation data of Robustness study.

Concentration	298nm	300nm	302nm
3ug/ml	0.124	0.431	0.234
3ug/ml	0.129	0.423	0.224
3ug/ml	0.125	0.435	0.232
3ug/ml	0.122	0.433	0.235
3ug/ml	0.125	0.438	0.236
3ug/ml	0.124	0.428	0.231
SD	0.125	0.4313	0.232
%RSD	1.856	1.233	0.187
Mean	0.00232	0.00532	00.4336
%RSD	1.856	1.233	0.187

Table -6: Evaluation data of Ruggedness study.

Concentration ug/ml	Analyst-1	Analyst -2
4ug/ml	0.570	0.568
4ug/ml	0.572	0.571
4ug/ml	0.573	0.574
4ug/ml	0.569	0.570
4ug/ml	0.571	0.573
4ug/ml	0.578	0.569
Mean	0.5722	0.570
SD	0.0032	0.0024
%RSD	0.558	0.406

Table 7: Summary of Validation Parameters.

Sr. NO	PARAMETER	LAMOTRIGINE
1	Wave length Max.	300nm
2	Linearity(*ug/ml*) (n=6)	1-5*ug/ml*
3	Regression equation	Y=
4	Correlation coefficient (r^2)	0.999
5	Accuracy (%Recovery) (n=3)	100.2%
6	Precision Intra-day (%RSD) n=6 Inter- day (%RSD) n=6	 1.142 1.075
7	Robustness (%RSD)	0.4314
8	Ruggedness (%RSD)	0.557, 0.406

CONCLUSION

A new analytical method has been developed to be routinely applied to determine Lamotrigine in pharmaceutical dosage form. In this study, the developed procedure has been evaluated over the specificity, linearity, accuracy, precision and robustness, Ruggedness in order to ascertain the stability of the analytical method. It has been proved that it was specific, linear, precise, accurate and robust and stability indicating. Hence, the method is recommended for routine quality control analysis and also stability sample analysis.

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