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Development and validation of a stability indicating HPLC assay method for determination of Lamotrigine in tablet formulation

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ABSTRACT

The objective of the current study was to developed simple, precise and accurate isocratic reversed-phase stability indicating High Performance Liquid Chromatography (HPLC) assay method and validated for determination of lamotrigine in solid pharmaceutical dosage forms. Isocratic RP-HPLC separation was achieved on a Phenomenex Luna C8 (2) column (250 mm x 4.6 mm i.d., 5 µm particle size) using mobile phase of methanol- ammonium buffer (50:50, v/v) at a flow rate of 0.8 ml/min and the detection was carried out at 225 nm by using photo-diode array detector. The drug was subjected to oxidation, hydrolysis, photolysis and heat to apply stress condition. The method was validated for specificity, linearity, precision, accuracy, robustness and solution stability. The method was linear in the drug concentration range of 40-160 µg/ml with a correlation coefficient 0.9976. The precision Relative standard deviation (RSD) amongst six-sample preparation was 1.05 % for repeatability and the intermediate precision (RSD) amongst six-sample preparation was 0.85 %. The accuracy (recovery) was between 98.20 and 100.86 %. Degradation products produced as a result of stress studies did not interfere with detection of lamotrigine and the assay can thus be considered stability indicating.

Key words: Lamotrigine, Stability indicating assay, Method validation, Isocratic.

INTRODUCTION

Stress testing is a part of developmental strategy under the International conference on harmonization (ICH) requirements and is carried out under more severe conditions than accelerated conditions. These studies serve to give information on drug's inherent stability and help in the validation of analytical methods to be used in stability studies[1-3]. It is suggested that stress testing should include the effect of temperature, light, oxidizing agents as well as susceptibility across a wide range of pH values. It is also recommended that analysis of stability sample should de done through the use of a validated stability testing methods.

Lamotrigine is chemically 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine (Figure 1) and it's Molecular formula is $C_9H_7N_5Cl_2$, and Molecular weight is 256.09 gm/mole. Lamotrigine, an antiepileptic drug (AED) of the phenyltriazine class, is chemically unrelated to existing antiepileptic drugs, has been used successfully to treat essential trigeminal neuralgia (TN)[4-7].

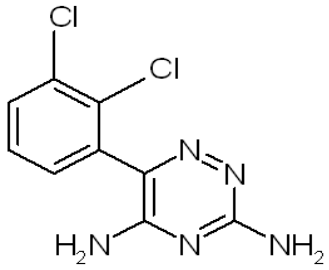

Figure: 1 Chemical structure of Lamotrigine

Some method have been reported like, liquid chromatography and UV spectrophotometric methods[8-10], capillary zone electrophoresis with some hyphenated techniques[11-12], adsorptive stripping voltammetry methods[13-14] and solid phase extraction method[15].

So far to our present knowledge, no validated stability indicating HPLC assay method for the determination of Lamotrigine in pharmaceutical formulation was available in literature. This paper deals with the forced degradation of Lamotrigine under stress condition like acid hydrolysis, base hydrolysis and oxidation, thermal and photolytic stress. This paper also deals with the validation of the developed method for the assay of Lamotrigine from its dosage form (tablets).

MATERIALS AND METHODS

Materials

Lamotrigine standard of was provided by Alembic Pharmaceuticals Ltd., Baroda (India). Lamotrigine tablets containing 25 mg Lamotrigine and the inactive ingredient used in drug matrix were obtained from market. Analytical grade ammonium acetate was purchased from Sisco Research Pvt. Ltd., Mumbai (India). HPLC grade methanol and water were obtained from Spectrochem Pvt. Ltd., Mumbai (India). Analytical grade hydrochloric acid, glacial acetic acid, sodium hydroxide pellets and 30% v/v hydrogen peroxide solution were obtained from Ranbaxy Fine Chemical, New Delhi (India).

Instrumentation

The chromatographic system used to perform development and validation of this assay method was comprised of a LC-10AT*vp* binary pump, a SPD-M10A*vp* photodiode-array detector and a rheodyne manual injector model 7725i with 20µl loop (Shimadzu, Kyoto, Japan) connected to a multi-instrument data acquisition and data processing system (Class-*VP* 6.13 SP2, Shimadzu).

Chromatographic conditions

Chromatographic analysis was performed on a Phenomenex Luna C8 (2) (250mm x 4.6mm i.d., 5 µm particle size) column. The mobile phase consisted of methanol – 0.01M ammonium acetate buffer pH 4.3 (50: 50, v/v). To prepare the buffer solution, 770.8 mg ammonium acetate were weighed and dissolve in 1000 ml HPLC grade water and then adjusted to pH 4.3 with glacial acetic acid. Mobile phase was filtered through a 0.45 µm nylon membrane (Millipore Pvt. Ltd. Bangalore, India) and degassed in an ultrasonic bath (Spincotech Pvt. Ltd., Mumbai). The flow

rate of the mobile phase was adjusted to 0.8 ml min^{-1} and the injection volume was 20 µl. Detection was performed at 225nm.

Standard preparation

A lamotrigine standard stock solution containing 250 µg/ml was prepared in a 200 ml volumetric flask by dissolving 50.00 mg of lamotrigine and then diluted to volume with methanol as a diluent and further takes 10 ml this stock solution in 50 ml volumetric flask and make up to mark methanol as a diluent. The concentration obtained was 50 µg/ml of lamotrigine.

Test preparation

Twenty tablets were weighed and the average weight of tablet was determined. From these, five tablets were weighed and transfer into a 500 ml volumetric flask. About 50 ml methanol was added and sonicated for a minimum 30 min. with intermittent shaking. Then content was brought back to room temperature and diluted to volume with methanol. The sample was filtered through 0.45µm nylon syringe filter. Take 10 ml this filtrate solution in 50 ml volumetric flask and make up to mark methanol as a diluent. The concentration obtained was 50 µg/ml of lamotrigine.

Method Validation

Specificity

The evaluation of the specificity of the method was determined against placebo. The interference of the excipients of the claimed placebo present in pharmaceutical dosage form was derived from placebo solution. Further the specificity of the method toward the drug was established by means of checking the interference of the degradation products in the drug quantification for assay during the forced degradation study.

Degradation study

The degradation samples were prepared by transferring powdered tablets, equivalent to 25 mg lamotrigine into a 250 ml round bottom flask. Then prepared samples were employed for acidic, alkaline and oxidant media and also for thermal and photolytic conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature and diluted with mobile phase to attain 50 µg/ml concentrations. Specific conditions were described as follows.

Acidic condition

Acidic degradation study was performed by heating the drug content in 20 ml 0.1 N HCl and 10 ml diluent at 80˚ C for 3 h and mixture was neutralized.

Alkaline condition

Alkaline degradation study was performed by heating the drug content in 20ml 0.1 N NaOH and 10 ml diluent at 80˚ C for 3 h and mixture was neutralized.

Oxidative condition

Oxidation degradation study was performed by heating the drug content in 20ml 30% v/v H_2O_2 and 10 ml diluent at 80˚ C for 3 h.

Thermal condition

Thermal degradation was performed by exposing solid drug at 80˚ C for 72 h.

Photolytic condition

Photolytic degradation study was performed by exposing the drug content in UV-light for 72 h.

Linearity
Linearity test solutions for the assay method were prepared at seven concentration levels from 40 to 160 % of assay analyte concentration (20, 30, 40, 50, 60, 70 and 80 µg/ml. The peak areas versus concentration data were evaluated by linear regression analysis.

Precision
The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of Lamotrigine test sample preparation and calculated the % RSD of assay (intraday). Intermediate precision of the method was checked by performing same procedure on the different day (interday) by another person under the same experimental condition.

Accuracy
An accuracy study was performed by adding known amounts of lamotrigine to the placebo preparation. The actual and measured concentrations were compared. Recovery of the method was evaluated at three different concentration levels (corresponding to 50, 100 and 150 % of test preparation concentration). For each concentration level, three sets were prepared and injected in duplicate.

Robustness of method
The robustness of study was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions. The factors chosen for this study were the flow rate (± 0.1 ml/min), mobile phase composition [methanol-buffer (48: 52 and 52: 48, v/v)], buffer pH (± 0.2 pH) and using different lot of Liquid chromatographic column.

Solution stability
The stability of solution for test preparation was evaluated. The solution was stored at ambient temperature and 2-5º and tested at interval of 12, 24, 36 and 48 h. The responses for the aged solution were evaluated using a freshly prepared standard solution.

RESULTS AND DISCUSSION

Development and optimization of the HPLC method
To develop a rugged and suitable HPLC method for the quantitative determination of lamotrigine, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration, organic solvent for mobile phase and mobile phase composition and other chromatographic conditions. Our preliminary trials using different composition of mobile phases consisting of water with methanol or acetonitrile, did not give good peak shape. By using 10mM ammonium acetate buffer, adjusted to pH 4.3 with glacial acetic acid and keeping mobile phase composition as methanol: ammonium acetate buffer (50:50, v/v), best peak shape was obtained. For the selection of organic constituent of mobile phase, methanol was chosen to reduce the longer retention time and to attain good peak shape. Figure 2 and Figure 3 represent the chromatograms of standard and test preparation respectively.

System suitability
A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate and % RSD of peak area were determined for same. For all system suitability injections, asymmetry was less then 2.0, theoretical plate was greater than 3500 and % RSD of peak area was less then 2.0 found.

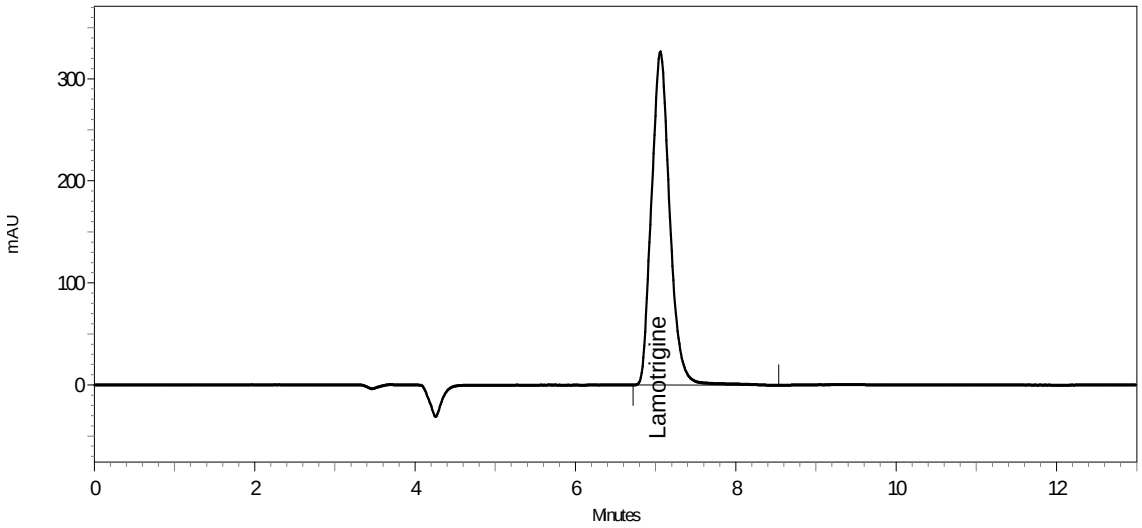

Figure 2: Chromatogram of standard preparation (Concentration 50 µg/ml and Wavelength 225 nm)

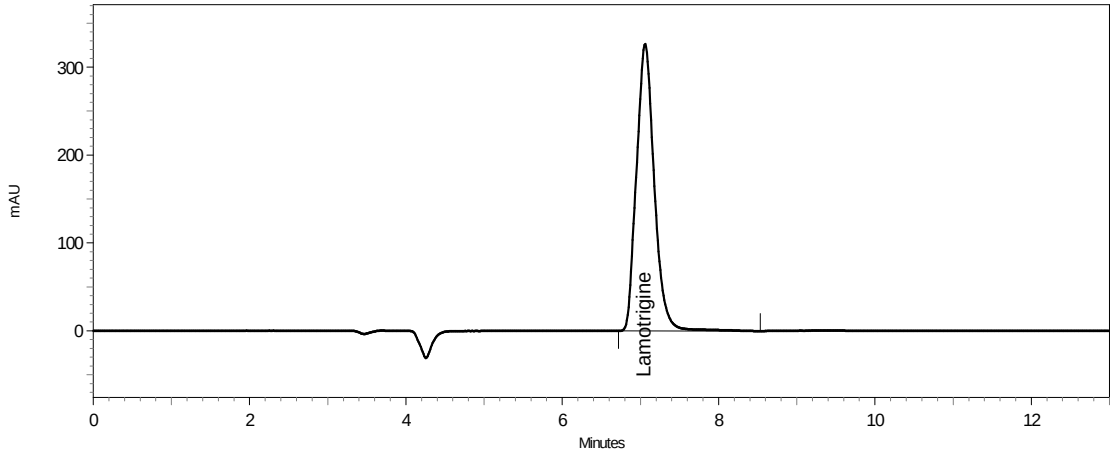

Figure 3: Chromatogram of test preparation (Concentration 50 µg/ml and Wavelength 225 nm)

Specificity

The specificity of the method was determined by checking the interference of placebo with analyte and the proposed method was eluted by checking the peak purity of lamotrigine during the force degradation study. The peak purity of the lamotrigine was found satisfactory under different stress condition. There was no interference of any peak of degradation product with drug peak. Major degradation was found in acidic condition that product was degraded up to 2.73 %. The major impurity peaks was found at 10.8 min Figure 4. In alkali degradation, it was found degraded up to 1.59 %. The major impurity peaks was found at 9.2 min Figure 5 and in photolytic condition around 0.18 % of the drug degraded Figure 6. Lamotrigine was found to be stable under the thermal degradation and oxidative condition.

Linearity

Seven points calibration curve were obtained in a concentration range from 20-80 µg/ml for lamotrigine. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y =132036853.57143x -76472.46 with correlation coefficient 0.9976.

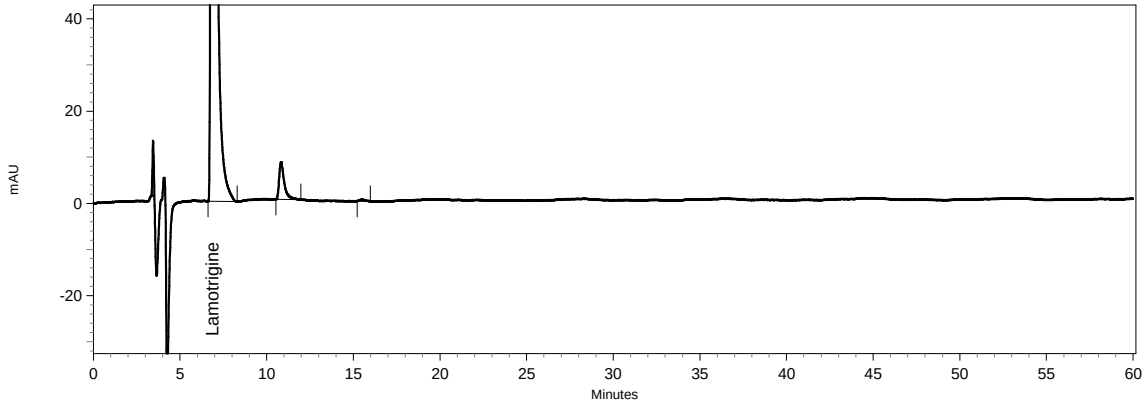

Figure 4: Chromatogram of acidic degradation study

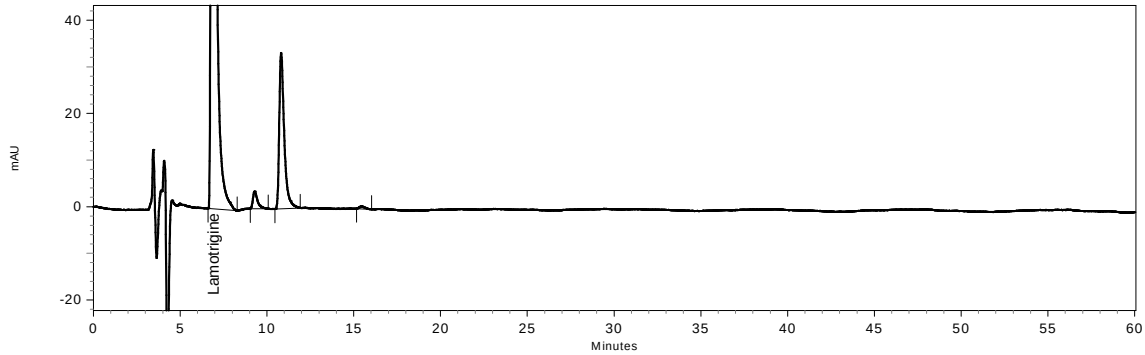

Figure 5: Chromatogram of alkali forced degradation study

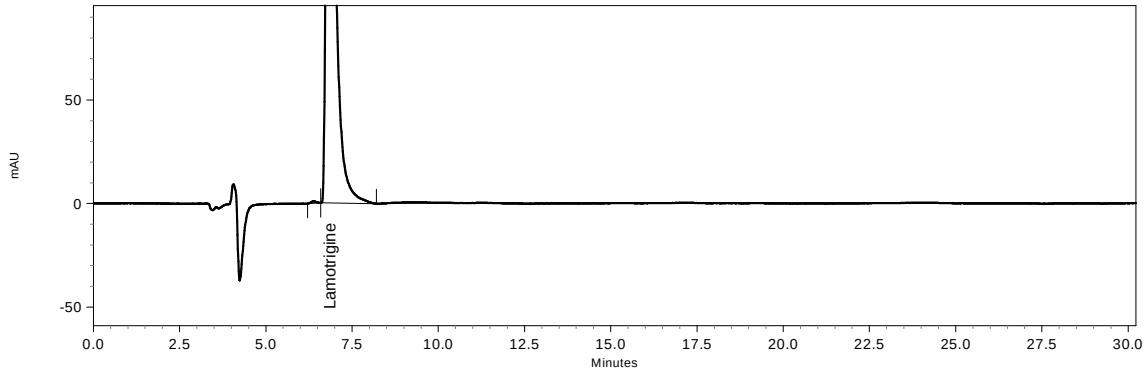

Figure 6: Chromatogram of photolytic degradation study

Table I: Evaluation data of precision study

Set	Intraday (n = 6)	Interday (n = 6)
1	98.8	98.2
2	98.1	99.4
3	99.4	100.3
4	98.8	98.8
5	98.1	98.5
6	99.8	98.1
Mean	98.8	98.9
Standard deviation	1.03	0.84
% RSD[a]	1.05	0.85

[a]*RSD= Relative Standard Deviation*

Precision

The result of repeatability and intermediate precision study are shown in Table 1. The developed method was found to be precise as the %RSD values for the repeatability and intermediate precision studies were < 1.05 % and < 0.85 %, respectively, which confirm that method was precise.

Accuracy

The HPLC area responses for accuracy determination are depicted in Table II. The results shown that best recoveries (98.02-100.86 %) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

Table II: Evaluation data of Accuracy study

Level (%)	Theoretical concentration (µg/ml)	Observed concentration (µg/ml)	% Recovery	% RSD
50	25.20	24.73	98.20	1.80
100	50.13	49.57	98.89	0.82
150	75.16	75.52	100.66	0.32

Solution stability

Table III shows the results obtain in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 48 h at 2-5˚ and ambient temperature as during this time the result was not decrease below the minimum percentage.

Table III: Evaluation data of solution stability study

Intervals	% Assay for test preparation solution stored at 2˚ C-8˚ C	% Assay for test preparation solution stored at ambient temperature
Initial	98.6	98.6
12 h	98.4	100.6
24 h	98.7	100.7
36 h	100.7	100.6
48 h	99.4	101.5

Table IV: Evaluation data of robustness study

Robust conditions	% Assay	System suitability parameters	
		Theoretical plates	Asymmetry
Flow 0.7 ml/min	99.5	3930	1.50
Flow 0.9 ml/min	99.6	3945	1.18
Buffer pH 4.1	100.7	3250	1.14
Buffer pH 4.5	100.5	4196	1.24
Buffer-Methanol (38:42,v/v)	100.6	3915	1.12
Buffer-Methanol (42:38,v/v)	99.4	3713	1.29
Column change	99.2	3730	1.28

Robustness of method

The result of robustness study of the developed assay method was established in Table IV. The result shown that during all variance conditions, assay value of the test preparation solution was

not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

CONCLUSION

A new analytical method has been developed to be routinely applied to determine lamotrigine in pharmaceutical dosage form. In this study, stability of lamotrigine in present dosage form was established through employment of ICH recommended stress condition. The developed procedure has been evaluated over the specificity, linearity, accuracy, precision and robustness 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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