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Research Article

DEVELOPMENT OF ANALYTICAL PROFILE OF LAMOTRIGINE AND ITS API FORMULATION

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ABSTRACT

Objective: The study is to highlight the development of lamotrigine and its derivative as an important pharmaceutical ingredient in many market formulations.

Methods: In the present work, one of the most imperative spectrophotometric methods, which is the RP-HPLC method, has been developed for the quantitative estimation of lamotrigine; in bulk and pharmaceutical formulations. UV spectrophotometric method, which involves the determination of lamotrigine; in bulk and bulk drug and pharmaceutical formulation, has maximum absorption at 307.5 nm in methanol. It obeys Beer Lambert's law in the concentration range of 5–45 µg/ml. A rapid and sensitive RP- HPLC Method UV detection (270 nm) for routine analysis of lamotrigine formulation was developed. Chromatography was performed using mobile phase containing a mixture of methanol and Phosphate buffer (65:35v/v) with a flow rate of 1.0 ml/min. In the range of 20–100 µg/ml, the linearity of lamotrigine and shows a correlation coefficient of 0.9998. The proposed method was validated by determining sensitivity and system suitability parameters.

Results: Along with the analytical profile of lamotrigine and its API formulation, various techniques have been explained successfully with proper demonstration of certain criterion.

Conclusion: The concept of lamotrigine and API formulation is a great tool which can lead to an excellent development of different types of formulation of lamotrigine.

Keywords: Lamotrigine, UV/Vis spectrophotometer, BMR, MBTH, HPLC.

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INTRODUCTION

Repeated administration of medication and its significant variations of drug level in the blood are common disadvantages of traditional dosing techniques of drug delivery. The frequency with which a rapidly absorbed and disseminated medication must be administered in a traditional dose form is determined by the drug's intrinsic characteristics, such as the elimination half-life (t1/2) [1]. Sustained-release dosage forms are intended to supplement the pharmaceutical activity of the drug to obtain better selectivity and longer duration of action, including improved therapeutic effect, increased patient compliance by reducing the frequency of dosing, and reducing the frequency and, or intensity of side effects caused by constant blood concentration [2]. An extended-release matrix tablet can be relatively easily prepared by incorporating drug particles into a matrix of slowly disintegrating or inert porous material containing a hydrophilic rate-controlling polymer [3-5]. Cellulose ethers such as hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC) and sodium carboxymethyl cellulose (Na CMC), acrylic methacrylic acid copolymers (Eudragits) such as Eudragit RL and RS, and some natural gums such as anthropic gum and gum, polymers are widely used as release retarders [6]. About 95% of all new potential therapeutic agents (APIs) show low and variable oral bioavailability due to their poor solubility in water at physiological pH and, consequently, low dissolution rate. According to the BCS, these drugs are classified as class II drugs, characterized by low solubility and high permeability, and represent significant challenges in developing pharmaceutical products. Lamotrigine, an anti-epileptic; drug approved in the United States to treat partial seizures and bipolar disorder, is classified in BCS Class II, and its oral bioavailability is approximately 98% [7,8]. It is commercially available as immediate-release and extended-release formulations in various dosages such as 25 mg, 50 mg, and 100 mg. In 2009, GSK obtained FDA approval for an extended version of lamotrigine (Lamictal) [9,10]. In this study, lamotrigine was chosen as a model drug and formulated as extended-release (SR) tablets to improve patient compliance. The chemical composition of lamotrigine is 6 (2,3 di-chlorophenyl) 1,2,4-triazine 3,5-diamine. Lamotrigine inhibits sodium currents by selectively binding to the inactivated state of the sodium channel and then inhibits the release of the excitatory amino acid, glutamate [11]. Therefore, the aim of this study aimed to prepare a prolonged-release lamotrigine tablet with the use of hydroxypropyl methylcellulose (HPMC) of various grades of K4M and K100M (their concentrations have been optimized) by wet granulation and with the use of various kinetic models to study the drug release mechanisms. Examine the effect of two independent variables (factors),that is, the amount of HPMC K4M and HPMC K100M, on the dependent variables, that is, T50%, t10%, k1 (time required to release 50% of the drug, time required to release 10% of the drug from the dosage form, first-order rate constant, respectively).

Molecular formula: $C_9H_7Cl_2N_5$
Molecular weight: 256.091
Chemical name: 3,5-Diamino-6-(2,3-dichlorophenyl)-as-triazine
Appearance: White to off white, Crystalline powder
Solubility: Soluble DMSO, Methanol, very slight soluble in water
Melting point range: 171–181
P-ka: 5.7
Therapeutic category: Broad-spectrum antiepileptic.

METHODS

Different reagents were used throughout the research project were of analytical grade purchased from reliable resources.

Chemicals used

Different chemicals with specificity were being used in it, such as lamotrigine Tablet (Standard Drug) with 99.9% Methanol, LAMETEC-100 DT Tablet, this tablet was the product of CIPLA company with batch no: SA93506 and manufacturing date of this tablet was August 19 and expiry date: July 21.

There are a few crucial things that must be taken for granted are as follows;

The temperature should not exceed more than 30°C. Distilled water must be appropriate as per standard rules. Furthermore, 100 ml of the calibrated volumetric flask (quantity-1) employed, Micropipette with a proper demonstration of working and calibrated 10 ml volumetric flask (quantity-10).

Method development of lamotrigine sustained-release tablets

The factorial design is a strategy for identifying and assessing the relative importance of elements involved in a process. Furthermore, any interactions between the components selected can be discovered. The creation of a factorial design includes the selection of parameters and the response options [12].

The percentage in which the independent variables HPMC K4M, HPMC K100M were utilized in the formulation of lamotrigine sustained-release (SR) Tablets was described using a three-level, two-factor experimental design (32 factorial design). The time necessary for 10% (t10%) and 50% (t50%) drug dissolution were chosen as dependent variables. For Final Equations, significance terms were chosen at a 95% confidence interval (p0.05). For t50%, t10%, and k1 (stepwise backward Linear Regression Analysis), polynomial equations were created.

The three concentrations of factor X1 (HPMC K4M) are 7.5%, 12.5%, and 17.5%. The design of the lamotrigine SR tablet formulation was based on three levels of factor X2 (HPMC K100M) at concentrations of 7.5%, 12.5%, and 17.5% (percentage of total tablet weight). A total of nine lamotrigine sustained release tablet formulations were created using 32 Factorial and evaluated to determine the significance of the combined effects of X1, X2 to choose the best combination and concentration required to achieve the desired prolonged/sustained release of drug from the dosage form.

Preparation of lamotrigine sustained-release tablets

All the ingredients were weighed accurately and passed through 40# sieve, blended in a Polybag except magnesium stearate for 10 minutes. The obtained mixture was wet massed using water (qs) for granulation and was passed through 20# sieve to form granules. These granules were dried and were passed through 30# sieve. These dried granules were lubricated with magnesium stearate, which was previously passed through 60# Sieve. The lubricated granules were punched into tablets using a rotary tablet punching machine (RIMEK), Ahmedabad).

Methodology used

UV spectrophotometric method

UV spectrophotometric determination of lamotrigine in Methanol.

Instrument

For this above-given method, Shimadzu model 1700 double beam UV-VIS spectrophotometer with a spectral bandwidth of 1.8 nm, wavelength accuracy of 2 nm, and a pair of 1 cm matched quartz cells of 10 mm optical path length was used for all spectral measurements.

Reagent used

Ammonium sulfamate (0.1%), (Bratton- Marshall Reagent) BMR (0.2%w/v), Conc. HCl, Ferric Chloride (0.2%, 0.3%, 1.62% and 0.5%w/v), HCl (1M, 2N, 4N), $NaNO_2$ (0.1% w/v), Potassium ferricyanide (0.1% w/v), (3-methyl-2-benzothiazolinone hydrazone hydrochloride) MBTH (0.2% w/v), NaOH (0.5% w/v), Double Distilled water.

Preparation of working standard solution for the above-given method

About 100 mg of standard lamotrigine was weighed and transferred into 100 ml volumetric flasks and dissolved in 50 ml of methanol, and then the volume was made up to the mark with methanol to obtain a final concentration of 1000 µg/ml (stock 'A' solution).

From the above stock, "A" solution, 10 ml of aliquot was pipetted out in a 100 ml volumetric flask, and the volume was made up to mark with methanol to obtain the final concentration of 100 µg/ml (stock "B" solution).

Preparation of working sample solution for the above-given method

Twenty tablets were powdered, and an amount equivalent to 100 mg of lamotrigine was accurately weighed and dissolved in 50 ml of methanol in a 100 ml volumetric flask and kept for ultrasonication for 5 min. It was diluted up to the mark with methanol to obtain 1 mg/ml solution. The solution was filtered through Whatman filter paper No.41, and this solution was used as stock, "A" solution.

From the above stock 'A' solution, 10 ml of aliquot was pipetted out in a 100 ml volumetric flask, and the volume was made up to the mark with methanol to obtain the final concentration of 100 µg/ml (stock "B" solution).

UV spectrophotometric determination of lamotrigine in methanol

It involves the determination of lamotrigine in bulk drug and pharmaceutical formulation. The absorption maximum was found at 307.5 in methanol (Fig. 1). It obeys Beer's laws in 5–45 µg/ml (Fig. 2).

Assay procedure

Aliquots of standard solution (stock B) of lamotrigine ranging from 0.5 to 4.5 ml (1ml = 100 µg) were transferred into a series of 10 ml with methanol and the absorbance was measured at 307.5 nm

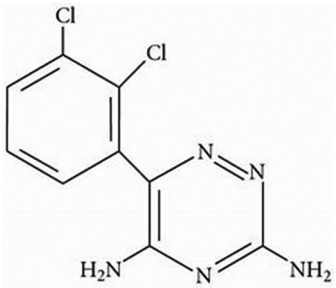

Fig. 1: Structural detail of lamotrigine

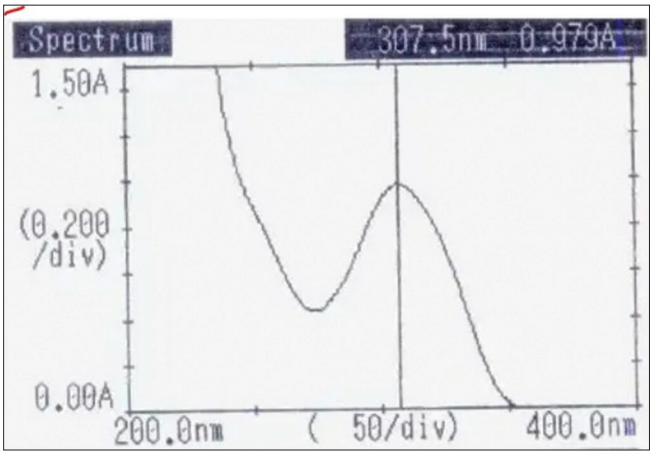

Fig. 2: Absorption spectrum of lamotrigine in methanol

against solvent blank. The obtained absorbance values, when plotted against the concentration of lamotrigine give the calibration graph. The concentration of the unknown sample was determined from the calibration graph or computed from the regression equation derived from Beer's law data.

Recovery studies

The study was to evaluate validity and reproducibility of the methods, recovery studies were carried out at the three different levels that are 80%, 100%, 120%, by adding the pure drug to the pre-analysed tablet powder sample as per ICH guidelines three times (n=3) (Fig. 3).

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Instrument

The specifications of the HPLC instrument used are as follows. A gradient high-pressure liquid chromatography (Shimadzu, class VP- Series) with one LC-10 AT VP pumps, UV/VIS detector SPD- 10A VP, CTO- 10 AS VP column oven (Shimadzu), a disposable guard column LC-18 (PELLIGUARD)™, LC-18, 2 cm, super, inc., Bellefonte, and a Reverse Phase C-18 Column (150mm by 4.6 mm i.d., particle size 5 micrometer) was used the HPLC system was equipped with software class, N- 2000 CHROMTECK (Shimadzu).

Chemicals and reagents

Methanol (HPLC grade)
Phosphate buffer (pH=4.5)
Double distilled water.

Preparation of standard stock solution of lamotrigine

About 100 mg of standard lamotrigine was accurately weighed and transferred to a 100 ml volumetric flask, dissolved in 10 ml of methanol, and diluted to 100 ml with buffer (pH-4.5), then sonicated for 10 min. From this, standard working solution of 100 µg/ml was prepared. Using the operational standard, aliquots of 20, 40, 60, 80, and 100 µg/ml were prepared with the buffer of pH (4.5). 20 µL of each dilution was injected into the column with a flow rate of 1ml/min.

Assay of lamotrigine in tablets

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 100 mg of lamotrigine was transferred to a 100 ml volumetric flask containing 10 ml of methanol and the contents of the drug, the mixture was then made up to 100 ml with the buffer of pH (45).

The resulting solution was thoroughly mixed and filtered through a 0.45 µm membrane filter. From this standard working solution of 100 µg/ml of strength was prepared. Aliquots of 20, 40, 60, 80, and 100 µg/ml were made in 100 ml volumetric flasks and diluted with a buffer of pH (4.5). This solution (20 µL) was injected five times into the column. The mean values of peak areas were calculated, and the drug content in the tablet was quantified using the regression equation.

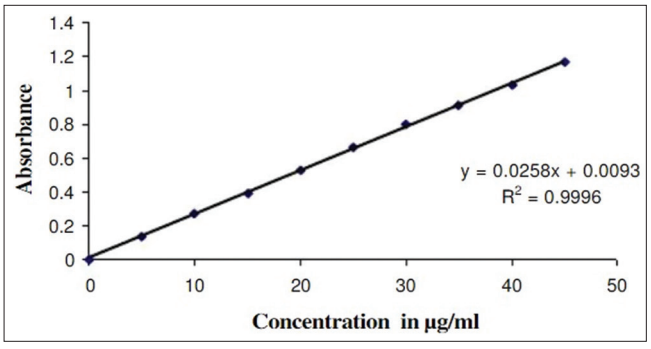

Fig. 3: Calibration curve of lamotrigine in methanol

Chromatographic condition

The content of the mobile phase was phosphate buffer solution (pH: 4.5) and methanol in the ratio of 65:35% v/v. The mobile phase was filtered through a 0.45 µm-membrane filter and sonicate for 15 min. The flow rate of the mobile phase was maintained at 1.0 ML/min. The column temperature was set at 20±1°C, and the detection was carried out by UV-detector wavelength at 270 nm. The run time was set at 10 min, and the volume of the injection loop was 20 µL. Before injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The data was acquired, stored, and analyzed with the software class N-2000 CHROMTECH (SHIMADZU).

The chromatogram obtained through the injection of the placebo solution did not contain any other peak at the retention time of lamotrigine. The chromatogram peak purity tools show that the rise was 100%. Thus, it was revealed that the peak at 3.743 min was not due to any interference from the excipients in the formulation (Figs. 4-6).

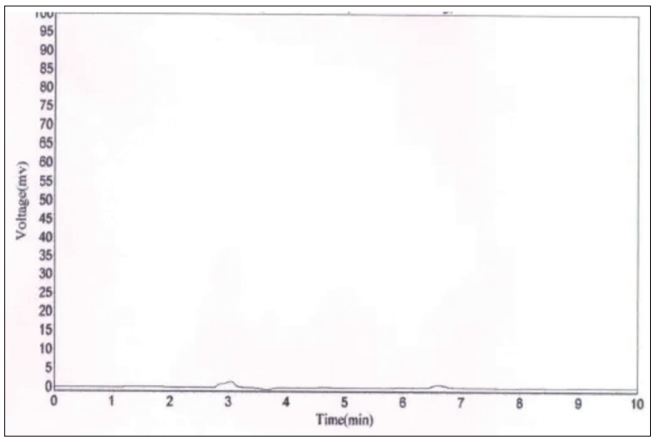

Fig. 4: Chromatogram of placebo solution of lamotrigine

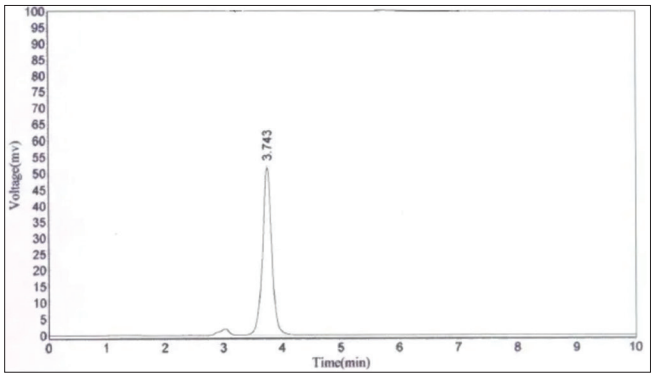

Fig. 5: Chromatogram of lamotrigine solution

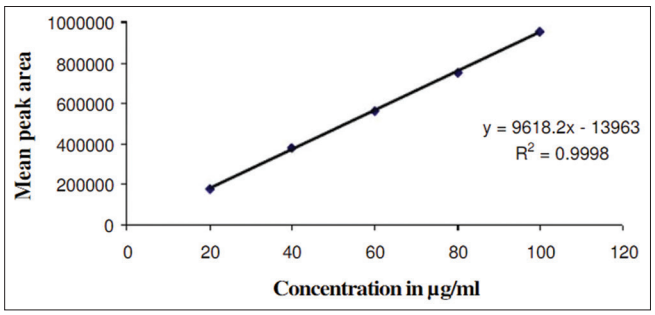

Fig. 6: Calibration curve of lamotrigine by RP-HPL

Table 1: Optical characteristics and precision of lamotrigine with methanol

Parameters	Methanol
λ max (nm)	307.5
Beer's law limits (µg/ml) (C)	5–45
Molar absorptivity (litre.mole^{-1} cm^{-1})	0.745 by 10^3
Sandell's sensitivity (µg/cm^2) 0.001 absorption units)	0.03816
Regression equation (Y*)	
Slope	0.0258
Intercept	0.0093
Correlation coefficient (r)	0.9996
% RSD	0.3440
Range of errors**	
Confidence limit with 0.05 level	0.0013
Confidence limit with 0.01 level	0.0020
LOD (µg/ml)	0.0305
LOQ (µg/ml)	0.0925

Y=bC+a, where C is the concentration of lamotrigine in µg/ml and Y is the absorbance at the respective λ max.

Table 2: Assay of lamotrigine by methanol

Formulation	Label claim (mg)	Concentration found (mg)	% Assay*
LAMITTOR	100	99.88	99.88
DT Tablet	100	99.80	99.80
	100	99.82	99.82

RESULTS

UV/visible spectrophotometry method [Tables 1 and 2]

To recapitulate, the above given tables which perceive the overall data of the research shows different parameters of lamotrigine and its API formulation with distinct values. In table 1 optical characteristics of Lamotrigine are given with methanol. In which, maximum wavelength, beers law limits, molar absorptivity, regression equation, slope, intercept, correlation coefficient, range of errors, LOD and LOQ are given.

In table 2 assay of lamotrigine by methanol has given with formulation, label claim, concentration and assay percentage.

Overall information of tables and graphs assist to perceive the development of analytical profile of lamotrigine and its API formulation.

CONCLUSION

A few medications are accessible as drug plans to control illnesses. Strategies for examining the centralization of these chemicals in the medicine and the living body are essential. The pharmaceutical analysis involves a crucial job in legal accreditation of drugs and their formulations either by the business or administrative specialists. The intricacy of issues experienced in drug examination combined with the significance of accomplishing the selectivity, speed, cost, effortlessness, and accuracy, and exactness results in new techniques for the investigation being immediately embraced by the drug industry. The steadily expanding utilization of pharmacodynamic and chemotherapeutic specialists in pharmaceutical arrangements makes their assurance an issue of foremost importance. Sometimes, no exact logical strategies are accounted for, and very often, the announced strategies need improvement or changes keeping considering the advances. Among a few instrumental procedures (HPLC, GLC, fluorimetry, NMR, mass spectrometry covering IR, UV, and noticeable locales) accessible for the examiner of medications, apparent spectrophotometric techniques rely on the idea of chemical reaction used for shading improvement and not on the refinement of the equipment. HPLC is a flexible apparatus for the subjective and quantitative examination of medications and pharmaceuticals, synthetic and natural materials, and has become vital in pharmacokinetics contemplates. HPLC strategy has been viewed as the best among various instrumental ones despite its weighty coast and upkeep problems. Due to the significance of examination, present insightful techniques have been developed for a portion of the broadly utilized anti-epileptic medication, for example, lamotrigine. Hence, we intended to create both HPLC and spectrophotometric methods.

AUTHORS CONTRIBUTION

All authors have contributed equally.

CONFLICTS OF INTEREST

None.

AUTHORS FUNDING

None.

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