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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF LEVETIRACETAM IN BULK AND PHARMACEUTICAL FORMULATION BY RP-HPLC

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ABSTRACT

This study describes the development and validation for levetiracetam tablets using a reverse phase high performance liquid chromatography method. A simple, reproducible and efficient reversed phase high performance liquid chromatographic (RP-HPLC) method has been developed for estimation of anti-epileptic drug, levetiracetam in raw material and its tablet dosage form. Separation was done by using mobile phase consisting of HPLC grade methanol. The separations were carried out on a Flexit C_{18} column (150 x 4.6 mm; 5µm) at a flow rate of 1 mL/min. The injection volume was 20 µl and the peaks were detected at 265 nm. The linear dynamic response was found to be in the concentration range of 5µg-25µg/mL and coefficient of correlation was found to be 0.999. The %RSD value was below 2.0 for intraday and interday precision indicated that the method was highly precise. The LOD and LOQ were found to be 4.23 and 12.65 ng/mL respectively which revealed that the method was highly sensitive. The percentage recovery value was higher than 100%, indicating the accuracy of the method and absence of interference of the excipients present in the formulation. The proposed method was simple, fast, accurate, precise and reproducible and hence can be applied for routine quality control analysis of levetiracetam in bulk and pharmaceutical formulation.

Keywords: Levetiracetam, RP-HPLC, anti-convulsant drug, method validation, linearity

INTRODUCTION

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Analytical chemistry is a subdiscipline of chemistry that has the broad mission of understanding the chemical composition of all matter and developing the tools to elucidate such compositions **[1-3]**.

HPLC is a liquid chromatographic separation technique conducted in high pressure where the sample (mixture constituents) is separated into its individual components by passing the sample present in a mobile phase (a flowing liquid) into a stationary phase (sorbents packed inside a column). Separation is due to the difference in affinity of individual components with mobile phase and stationary phase. The basic components are solvent delivering unit, sample injector, column and a detector **[4-6]**.

Once the analytical method is developed, it is important to perform validation of an analytical method. ICH Q1b provides guidelines regarding the validation of analytical procedures. The method validation ensures that the developed analytical method is suitable for its intended purpose **[7]**. The validation parameters include multiple parameters. However, in the initial stage of development, it is not mandatory to perform all the validation studies. During phase II (preliminary efficacy) trials, researchers focus on specificity, linearity, accuracy, and precision studies and the remaining studies are performed when the drug passes to phase III (efficacy) stage of development and has a higher probability of becoming a marketed product **[8-11]**.

Drugs are inevitable part of our life. We use the drugs since the offspring borne till it dies. The quality of the drugs is an essential feature as it directly affects the life of consumers. The quality of any product or material can be best judged by analysing it. Analysis is a branch of science and it deals with the qualitative and quantitative measurement of any matter. Analysis can give us the answer what (Qualitative analysis) is present and how much (Quantitative analysis) is present in the matter **[12]**. Analysis is found in almost every branch whether it is data analysis, market analysis or pharmaceutical analysis. Among these the pharmaceutical analysis deals with the quality of pharmaceutical products and ultimately life of the consumer. Another requirement for quality drug is that the regulatory and government agencies became stringent in case of poor-quality drug products. Quality of any drug product can be known by a series of tests starting from the testing of raw material, intermediates and finished products etc. Drug analysis deals with the identification,

characterization and quantification of drugs in singular or in combination as in dosage forms, biological fluids and bulk drugs. As a pharmaceutical analyst we are interested in qualitative analysis of given drug(s) whether it is present in the given material and in quantitative analysis we are interested in the amount of drug(s) present in the given sample **[13-16]**.

Today's world is an arena where cutthroat competition is seen. People are going on making new drugs and new formulations of the existing drugs within very short period of time. To get the regulatory permission for marketing, company has to submit required data including the analysis reports as to prove that their drug product is of required quality for its intended use. For these new drugs and formulations there are no standard official procedures available for its analysis so, we need to develop some method for the identification and estimation of drugs **[17-20]**.

Levetiracetam is an anti-epileptic agent and chemically known as (S)-2-(2-oxopyrrolidin-1-yl) butanamide **(Figure 1)** and it has a molecular formula: $C_8H_{14}N_2O_2$, and molecular weight: 170.20g/mol. Levetiracetam is very soluble in water and freely soluble in methanol and chloroform. In n-hexane levetiracetam is insoluble practically. The exact mechanism through which levetiracetam exerts its anti-epileptic effects is unclear, but is thought to be unique amongst other anti-epileptic medications. Levetiracetam acts by modulating the synaptic neurotransmitter release and by binding itself to synaptic vesicle protein SV2A in the brain. Levetiracetam was approved by the US FDA. Levetiracetam is absorbed completely after the oral administration and about 100% bio-availability was reported. It undergoes metabolism through enzymatic hydrolysis of acetamide group. Levetiracetam maintains a large margin of safety and no interactions were reported with other anti-convulsant and due to this levetiracetam is used as an adjunctive therapy for the treatment of epileptic seizures **[21-23]**.

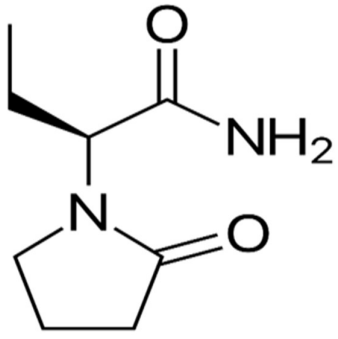

Figure 1: Molecular structure of levetiracetam

Figure 2: HPLC used

Various UV, HPLC and LC-MS methods have been reported for the determination of levetiracetam. A literature survey revealed that very few HPLC methods have been reported for the determination of levetiracetam from pharmaceutical dosage form. Hence, in this present investigation an attempt has been made to develop an accurate, precise and economically viable reversed phase HPLC method for the estimation of levetiracetam in bulk drug and in pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals and reagents

Levetiracetam (API) was obtained as a gift sample from Hetero Labs Ltd., Hyderabad, Telangana. The commercially available levetiracetam tablets claimed to contain 250 mg of active ingredients were procured from local pharmacy, Hyderabad. All the chemicals and reagents used were analytical grade. Methanol, acetonitrile and distil water used were of HPLC grade.

Apparatus and chromatographic condition

The chromatographic separation was performed on a Prominence Shimadzu high performance liquid chromatographic instrument equipped with a Flexit C_{18} column (150 x 4.6mm; 5µm) integrated with UV detection at 265 nm. The mobile phase consisting of HPLC grade methanol and was prepared freshly, filtered and sonicated before use and delivered at a flow rate of 1 mL/ min. The volume of each injection was 20µl. The column and the HPLC system were kept in ambient temperature. The experiment was performed at room temperature.

Selection of solvent [24]

A number of trails were made to find out the ideal solvent for dissolving the drug. The solvents such as HPLC grade distil water,

methanol and acetonitrile were tried based on the solubility of the drug.

Detection of wavelength (λ_{max}) [25-27]

Appropriate dilutions of levetiracetam were prepared from the standard stock solution. The standard solution of levetiracetam in distil water was scanned over wavelength range 200 to 400 nm by using UV-Visible spectrophotometer.

Preparation of stock solution [28]

Stock solution of levetiracetam was prepared by dissolving 100 mg of levetiracetam in 100 mL of standard volumetric flask containing 25 mL of mobile phase and the solution was sonicated for 20 min and then made up to the mark with mobile phase to get a concentration of 1 mg/mL. Subsequent dilutions of this solution were made with mobile phase to get concentrations of 40-400 µg/mL. The standard solutions prepared as above were injected into the 20 µL loop and the chromatogram was recorded.

Analysis of formulation (tablets) [29]

Ten tablets each claimed to 250 mg of levetiracetam were weighed accurately and powdered. A quantity equivalent to 100 mg of levetiracetam was weighed accurately and transferred to a 100 mL volumetric flask. Then 25mL of the mobile phase was added to it and the mixture was sonicated for 20 min and then diluted up to the mark with the same solvent. The resulting solution was filtered through a membrane filter. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity as previously discussed for the pure drug. Sample solution was injected under the chromatographic conditions as mentioned above and the chromatogram was recorded.

Validation for the developed method [30-31]

The aim of method validation was to confirm that the present method was suitable for its intended purpose as prescribed in ICH guidelines. The method was validated in order to determine the system suitability, linearity, precision, accuracy, repeatability, ruggedness, LOD and LOQ of the method.

System suitability test

The system suitability tests were carried out on freshly prepared standard stock solution of levetiracetam to evaluate the suitability of the system and the parameters that were studied presented in Table 1. From the typical chromatogram of levetiracetam as shown in Figure 5, it was found that the average retention time ± standard deviation for levetiracetam was found to be 5.192±0.001 min for five replicate injections. The asymmetry factor was found to be 1.78, which indicated asymmetric nature of the peak. The number of theoretical plates was found to be 3346, which suggested an efficient performance of the column. The absence of additional peaks in the chromatogram indicated non-

interference by the common excipients used in the tablet formulation.

Linearity

The linearity of this method was determined at five concentration levels ranging from 5µg/mL 25µg/mL. The plot of peak area of each sample against respective concentration of levetiracetam was found to be linear in the range of 5-25 µg/mL. Beer's law was found to be obeyed over this concentration range.

Precision

The precision is a measure of the ability of the method to generate reproducible results. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as %RSD. For this, 120 µg/mL of the solution was measured three times in a day and the same was repeated in next three days. The precision (measurements of intraday and interday) results showed good reproducibility with percent relative standard deviation (% RSD) was below 2.0%. This indicated that method was highly precise.

Recovery studies (Accuracy)

Recovery studies were performed to judge the accuracy of the method. The studies were carried out by adding a known quantity of pure drug to the pre-analyzed formulation and the proposed method was followed. From the amount of drug found, the percent recovery was calculated. Recovery study was carried out at three levels 80%, 100% and 120% for the formulation concentration of 120µg/mL. The percentage recovery value, which was higher than 100 %, indicated that the accuracy of the method and absence of interference of the excipients present in the formulation.

Robustness

Robustness was performed by small but deliberate variation in the chromatographic conditions and was found to be unaffected by small variations like ±2% in volume of mobile phase composition, ±0.1 mL/min in flow rate of mobile phase and ±1% change in column temperature. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

Limit of detection (LOD) and limit of quantification (LOQ)

Limit of detection (LOD) and limit of quantification (LOQ) were calculated by the using the equation given in ICH guidelines. This may be expressed as LOD = 3.3 σ /S and LOQ = 10 σ/S, where σ is the standard deviation of the response, S is the slope of the calibration curve which may be estimated from the calibration curve of the analyte. The LOD and LOQ for levetiracetam were found to be 4.23 ng/mL and 12.65 ng/mL respectively (Table 4), this demonstrated that the method was highly sensitive.

RESULTS AND DISCUSSION

Validated analytical methods are aimed for the determination of levetiracetam in API and its formulation. All of the analytical validation parameters for the proposed method were determined according to ICH guidelines.

Absorbance maxima (λ_{max})

The absorbance maximum of levetiracetam was found to be 265 nm in methanol. The UV spectrum for levetiracetam is depicted in **Figure 1**.

Method validation

The proposed method was validated as per ICH Q2 guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experimental work.

Linearity

The regression equation was found to be Y = 376115x + 1E + 07 and the correlation coefficient (r) of the standard curve was found to be 0.999 **(Table 2, Figure 4)**.

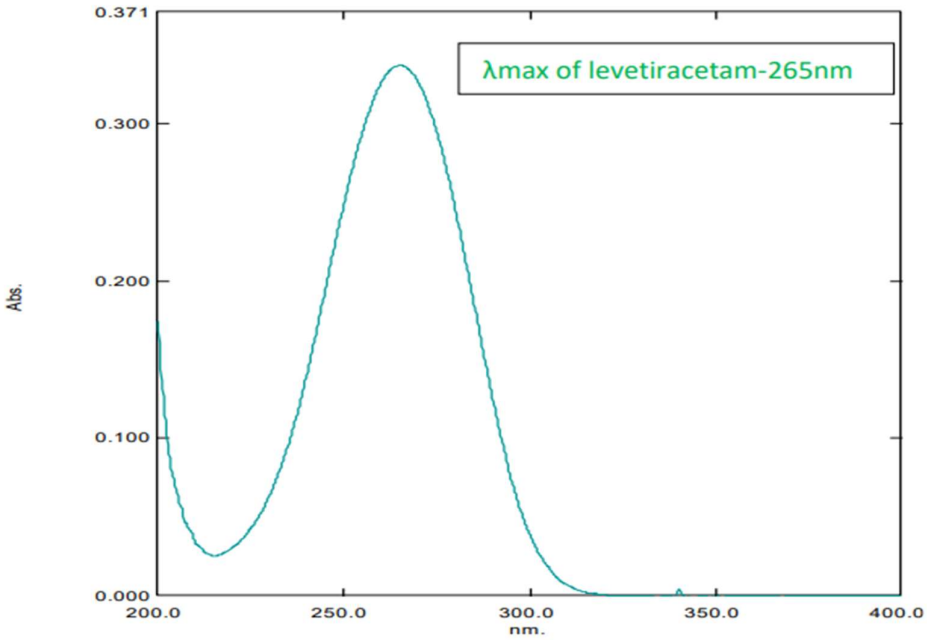

Figure 3: Absorption spectrum of levetiracetam

Table 1: System suitability parameters

Retention time (min) ± S.D.	**5.192± 0.001**
No. of theoretical plates	**3346**
Asymmetric factor	**1.78**

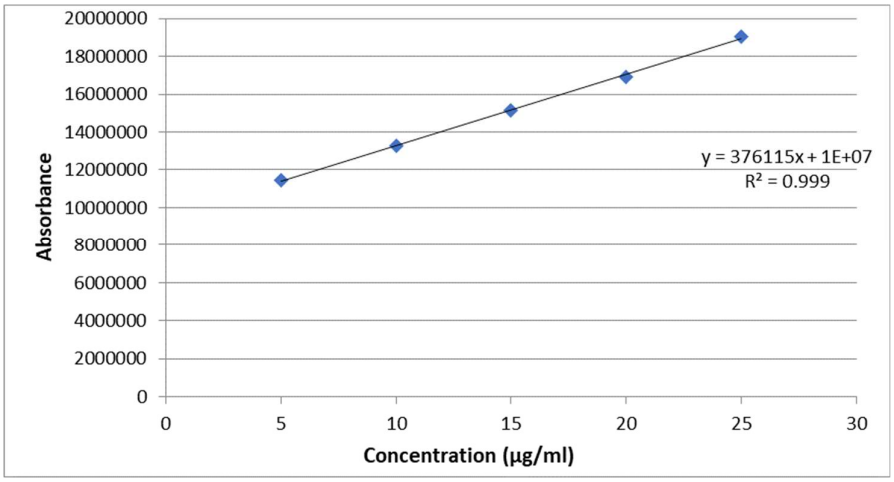

Figure 4: Calibration curve of levetiracetam

Table 2: Recovery

Level of Addition (%)	Formulation (µg/mL)	Addition of pure drug (µg/mL)	% Recovery of pure drug	Recovery (%) ± S.D.
80	120	96	101.34	101.55±0.37
100	120	120	101.33	
120	120	144	101.99	

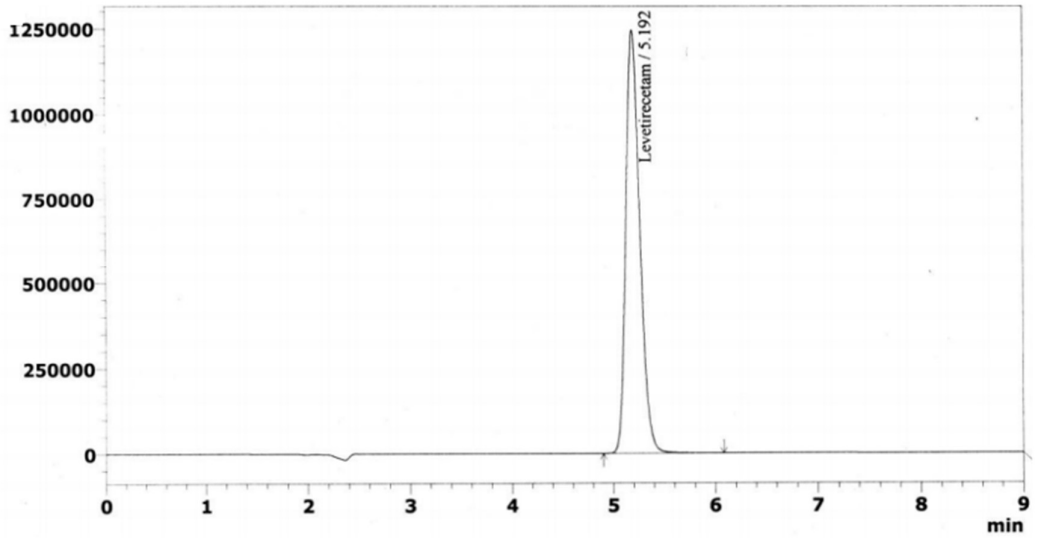

Figure 5: Typical chromatogram of levetiracetam

Table 3: Validated parameters

Parameters	Results
λ_{max}	265 nm
Linearity range (µg/ml)	5 - 25
Standard Regression equation	Y = 376115x + 1E + 07
Correlation coefficient	0.999
Precision (at 120µg/ml)	Intraday (% RSD) – 0.354
	Interday (%RSD) – 0.265
LOD (ng/ml)	4.23
LOQ (ng/ml)	12.65

Table 4: Assay of formulation (tablets)

Drug	Label claim	Amount present	% Amount present
Levetiracetam	250mg	252.72±0.67	101.09

A comprehensive summary of the work to be incorporated in the thesis entitled "Analytical method development and validation for the determination of levetiracetam in bulk and pharmaceutical formulation by RP-HPLC" has been describe as under, the research work undertaken in these studies mainly addresses analysis, development of stability indicating HPLC methods and validation protocol, according to ICH guidelines.

The proposed HPLC analytical method for the quantification of levetiracetam in API and tablet formulation is simple, accurate, and rapid and can be employed for the routine analysis. Once the absorbance of the sample is determined, it requires only simple calculation. This method can be applied for the substances which obey Beer's law. The low standard deviation and good percentage recovery indicated the reproducibility and accuracy of the method.

Because of cost-effective and minimal maintenance, the present RP-HPLC method can be preferred at small scale industries and successfully applied and suggested for the quantitative analysis of levetiracetam in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

Many pharmaceutical industries manufacture their formulation of all mentioned drugs either in combination or in single dosage form. Most of the pharmaceutical industries use time consuming method and different mobile phases for different dosage form of drugs. But with the proposed method developed, time and cost required for changing different mobile phases could be saved, because only one mobile phase can be used for six drugs and their combinations. This makes the method suitable for routine analysis in quality control laboratories.

CONCLUSION

From present research work, it is concluded that it is economical and reproducible. The method was developed and validated as per ICH Q2 (R1) guidelines. The proposed methods can be employed for routine analysis of bisoprolol from pharmaceutical dosage form (Tablets). It is inferred that the methods were found to be simple, accurate, precise and linear. The methods were found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision. The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogeneous sample. The results showed that the recovery of marketed product by the proposed method was satisfactory. The validation procedure confirms that this is an appropriate method for their quantification in the plant material and formulation. It is also used in routine

quality control of the raw materials as well as formulations containing this entire compound. The result obtained from the validation parameters met the ICH Q2 and USP requirement as well as obeys Beer's law.

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Declarations

Author contributions

All authors contributed to experimental work, data collection, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Ethical approval

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