Research Article

Development and Validation of Novel HPLC Method for Estimation of Levetiracetam in Pharmaceutical Formulations

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ABSTRACT

A simple, rapid, accurate and precise RP-HPLC method was developed for the determination of of Levetiracetam in pharmaceutical formulations and in bulk materials using an isocratic Agilent LC 1100 series HPLC instrument on a wakosil RS C18 column (250 mm x 4.6 mm, 5µ). The method showed a linear response for concentration in the range of 120 – 360 µg/mL using potassium dihydrogen phosphate buffer (p^H 3) and methanol as the mobile phase in the ratio of 90:10 v/v with variable wavelength UV-Visible detector. Data was analysed by using Chemstation software. Elico SL 159 UV-Visible spectrophotometer was used for UV spectral studies and detection was carried out at 254nm with a flow rate of 1.5 mL/min and retention time was 15.406 min. The method was statistically validated for linearity, accuracy, precision and selectivity. Quantitative and recovery studies of the dosage form were also carried out and analyzed, the %RSD from recovery studies was found to be less than 1. Due to simplicity, rapidity and accuracy of the method, the developed method will be useful for routine quality control analysis of Levetiracetam in pharmaceutical formulations.

Keywords: Levetiracetam, Estimation, Tablets, RP-HPLC.

INTRODUCTION

Levetiracetam (Figure 1) is an analogue of piracetam. It is used as an adjunct in the treatment of partial seizures with or without secondary generalizations in adults and children's aged 4 years and over. In addition, Levetiracetam is licensed for adjunctive use in the treatment of myoclonic seizures in the adults and children's aged 12 years and over with juvenile myoclonic epilepsy. It is also licensed for use as an adjunct in the treatment of primary generalized tonic-clonic seizures in adults and children's with idiopathic generalized epilepsy[1]. Levetiracetam acts by binding stereo selectively to synaptic plasma membrane in the brain and affects allostearic modulations of not only GABA receptors but of high voltage activated Ca^{2+} channels and K^{+} channels[2].

A few analytical methods have been reported for the determination of Levetiracetam in pure drug, pharmaceutical dosage forms and biological samples using spectrophotometry[3], liquid chromatography[4-18], gas chromatography[19], ion exchange chromatography[20], electrokinetic chromatography[21, 22], capillary electrochromatography[23], electrophoresis[24] and electrochemical method[25].

MATERIALS AND METHODS

Instrumentation

The author had attempted to develop and validate a liquid chromatographic method for the determination of Levetiracetam using an isocratic Agilent LC 1100 series HPLC instrument on a wakosil RS C18 column (250 mm x 4.6 mm, 5µ). The instrument is equipped with a binary pump and variable wavelength UV-Visible detector. A 20µL Hamilton syringe was used for injecting the samples. Data was analysed by using Chemstation software. Elico SL 159 UV-Visible spectrophotometer was used for UV spectral studies. Degassing of the mobile phase was done by using a Loba ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials.

Chemicals and Solvents

The reference sample of Levetiracetam API was obtained as gift sample from Ranbaxy laboratories limited, Gurgaon. The branded formulation of Levetiracetam tablets (Levroxa tablets containing 250 mg of Levetiracetam) were procured from the local market. Methanol, Water and orthophosphoric acid used were of HPLC grade and potassium dihydrogen phosphate AR grade were

purchased from Merck Specialities Private Limited, Mumbai, India.

Preparation of mobile phase
A mixture of potassium dihydrogen phosphate buffer (pH 3.0) and methanol in the ratio of 90:10 v/v was prepared and used as mobile phase.

Preparation of standard solution
About 240 mg of Levetiracetam standard was weighed and transferred into a 100 mL volumetric flask containing 60 mL of mobile phase. The solution was sonicated for 15 min and then volume was made up with further quantity of the mobile phase to get a concentration of 2.4 mg/mL solution. 10 mL of this solution was further diluted to 100 mL with mobile phase to get a concentration of 240µg/mL.

Preparation of (tablets) sample solution
Twenty tablets were weighed and finely powdered. An accurately weighed portion of this powder equivalent to 240 mg of Levetiracetam was transferred to a 100 mL volumetric flask containing 60mL of the mobile phase. The contents of the flask were sonicated for about 15 min for complete solubility of the drug and volume made up with further quantity of mobile phase. Then this mixture was filtered through whatman No.41filter paper. 10 mL of this filtrate was further diluted to 100 mL with mobile phase.

Procedure
A mixture of potassium dihydrogen phosphate buffer (pH 3.0) and methanol in the ratio of 90:10 v/v was found to be the most suitable mobile phase for ideal separation of Levetiracetam. The solvent mixture was filtered through whatman No.41filter paper and sonicated before use. It was pumped through the column at a flow rate of 1.5 mL/min. The column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. Inject 20 µL of the standard, sample solutions into the chromatographic system and measure the area for the Levetiracetam peak. The detection of the drug was monitored at 254nm. The run time was set at 20 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 15.406 min. A typical chromatogram showing the separation of the drug (Figure 2).

Calibration Plot
About 240 mg of Levetiracetam was weighed accurately, transferred into a 100 mL volumetric flask and dissolved in 60 mL of mobile phase. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the mobile phase. Further dilutions ranging from 120 - 360 µg/mL were prepared from the stock solution in 10 mL volumetric flasks using the above diluent. 20 µL of each dilution was injected six times into the column at a flow rate of 1.5 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 120 - 360 µg/mL of the drug. The relevant data are furnished in Table-1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of Levetiracetam in tablet dosage forms.

Validation of the proposed method
The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method as per the ICH guidelines for the estimation of Levetiracetam [26]. Solution containing 2400 µg/mL solution of Levetiracetam was subjected to the proposed HPLC analysis to check system precision, method precision and intermediate precision of the method and the results are furnished in Table-2 to Table-4. The accuracy of the HPLC method was assessed by analyzing solutions of Levetiracetam at 50, 100 and 150% concentration levels by the proposed method. The results are furnished in Table-5. The system suitability parameters are given in Table-6.

Estimation of Levetiracetam in tablet dosage forms
Commercial formulation of tablets was chosen for testing the suitability of the proposed method to estimate Levetiracetam in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 240 mg of Levetiracetam was transferred into a 100 mL volumetric flask and dissolved in 60 mL of mobile phase. The contents of the flask were sonicated for 15 min continuously to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through whatman No.41filter

paper. This solution containing 240 µg/mL of Levetiracetam was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table-5.

DISCUSSION

The present study was aimed at developing a simple, sensitive, precise and accurate HPLC method for the analysis of Levetiracetam from tablet dosage forms. A non-polar C_{18} analytical chromatographic column was chosen as the stationary phase for the separation and determination of Levetiracetam. Mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different combinations were tested as mobile phases. The choice of the optimum composition is based on the chromatographic response factor, a good peak shape with minimum tailing. A mixture of buffer and methanol in the ratio of 90:10 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was well defined, better resolved and almost free from tailing. The retention time of the drug was found at 15.406 min.

A good linear relationship (r = 0.9999) was observed between the concentration of Levetiracetam and the corresponding peak areas. The linearity was found satisfactory in the range 120 – 360 µg/mL (Table 1). The regression equation of the linearity curve between concentration of Levetiracetam over its peak area was found to be Y = 13621.91X + 17030.16 (where Y is the peak area and X is the concentration of Levetiracetam in µg/mL). Precision of the method was studied by repeated injection of Levetiracetam tablet solution and results showed lower %RSD values (Table 2, 3 and 4). This reveals that the method is quite precise. The percent recoveries of the drug solutions were studied at three different concentration levels. The percent individual recovery and the %RSD at each level were found within the acceptable limits (Table 5). This indicates that the method is accurate. The absence of additional peaks in the chromatogram indicates non-interference of the commonly used excipients in the tablets and hence the method is specific.

The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust . The system suitability studies were carried out to check various parameters such as theoretical plates and tailing factor. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive (Table 6). The solution stability studies indicate that the Levetiracetam drug was stable up to 24 hours.

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Levetiracetam and can be reliably adopted for routine quality control analysis of Levetiracetam in its tablet dosage forms.

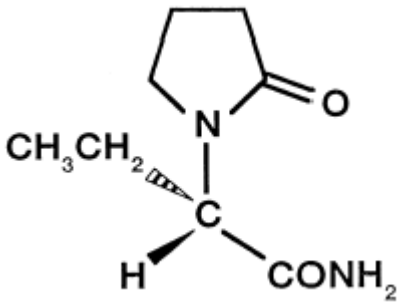

Fig. 1: Molecular structure of Levetiracetam

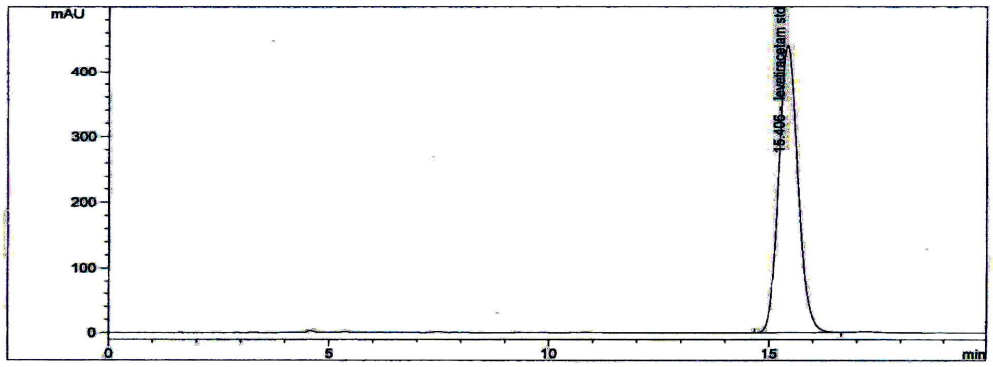

Fig. 2: Chromatogram of Levetiracetam standard

Table 1: Linearity study

Concentration of Levetiracetam (µg/mL)	Mean peak area
120	1663086
192	2643459
216	2968289
240	3310816
264	3599395
288	3921185
360	4914825

Table 2: System precision

Injection number	Peak area of Levetiracetam
1	3268262
2	3267558
3	3267747
4	3268910
5	3271965
6	3271338
%RSD	0.06

Table 3: Method precision

Preparation number	% Assay of Levetiracetam
1	96.26
2	95.26
3	96.45
4	96.45
5	96.73
Mean	96.33
%RSD	0.58

Table 4: Intermediate precision

Injection number	% Assay of Levetiracetam
1	96.24
2	97.01
3	96.43
4	96.77
5	96.98
6	96.52
Mean	96.66
%RSD	0.32

Table 5: Recovery studies

%Concentration (at specification Level)	Mean peak area	Amount of Levetiracetam added (mg)	Amount of Levetiracetam found (mg)	%Recovery	Mean % Recovery
50%	1663086	121	121.4	100.3%	100.0%
100%	3310816	241	241.2	100.1%	
150%	4914825	360	358.8	99.8%	

Table 6: System suitability parameters

Parameter	Result
Linearity ((µg/mL)	120 – 360
Correlation coefficient	0.999
Theoretical plates (N)	4763
Tailing factor	1.53
LOD (µg/mL)	0.52
LOQ (µg/mL)	1.16

Table 7: Results of Assay

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Tablet	250	249.6	99.69

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