RP-HPLC METHOD FOR THE ESTIMATION OF LEVETIRACETAM IN BULK AND PHARMACEUTICAL FORMULATIONS

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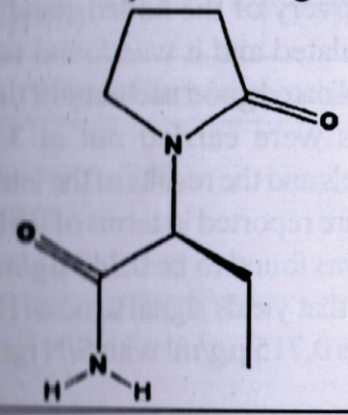
ABSTRACT

A simple, specific, accurate reverse phase liquid chromatographic method was developed for the determination of levetiracetam in bulk and in tablet dosage forms. A Waters C-18 column having 250×4.6 mm i.d., with mobile phase containing acetonitrile: 0.01 M potassium dihydrogen phosphate (10:90 v/v; pH 7.0) was used. The retention time of levetiracetam was 6.93 min. The linearity for levetiracetam was in the range of 40-240 µg/ml. The recovery was found to be in the range of 98.6-100.6%. The detection limit and quantification limit were found to be 0.347 µg/ml and 0.715 µg/ml respectively. The proposed method was validated and successfully applied to the estimation of levetiracetam in tablet formulations.

KEY WORDS: levetiracetam, reverse phase liquid chromatography, validation.

1.INTRODUCTION

Levetiracetam (www.Rxlist.com; Physician desk reference,2006) is a novel antiepileptic agent; with a chemical name (S)-(2)-(2-oxopyrrolidin-yl) butamide with a molecular formula $C_8H_{14}N_2O_2$ and a molecular weight of 170.20. It is used as an adjunctive therapy in the treatment of partial seizures (Rathnaraj and Doheny,1996). Literature survey reveals many chromatographic methods for the determination of levetiracetam in biological fluids (Jens,2005; Rao,2004;Tiegong,2007;Isoherranen,2000). So far, no assay procedure has been reported for the estimation of levetiracetam from pharmaceutical dosage form. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of levetiracetam in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reverse-phase HPLC method (Snyder,1997) for the estimation of levetiracetam in bulk drug samples and in pharmaceutical dosage form and validate the developed method as per the ICH guidelines (1996).



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2.MATERIALS AND METHODS

The liquid chromatographic system of Younglin make containing Variable wavelength programmable UV/Vis detector and Rheodyne injector with 10 µl fixed loop was used. Chromatographic analysis was performed using Autochro 3000 software. A waters C18 column with 250×4.6mm i.d. and 5 µm particle size was used. Acetonitrile, water (E. Merck, Mumbai, India) were of LC grade, while potassium dihydrogen phosphate (S. D. Fine Chemicals, Mumbai, India) was of analytical grade used for the preparation of mobile phase.

Preparation of mobile phase and stock solution

Potassium dihydrogen phosphate was weighed (0.34 g) and dissolved in 100 ml of water. The pH of the solution was adjusted to 7 with the help of 10% Potassium hydroxide. The solution was sonicated for 10 minutes and filtered using Whatman filter paper (No.1). The Stock solution was prepared by weighing (25 mg) and transferring analytically pure levetiracetam to 25 ml volumetric flask. Volume was made up to the mark with diluent (Acetonitrile and Water 10:90), which gave 1000 µg/ml of the drug. The solution was further diluted with the same diluent to obtain final concentration of 200 µg/ml.

Chromatographic conditions

A reverse phase C18 column equilibrated with mobile phase Acetonitrile: 0.01 M potassium dihydrogen phosphate (10:90 v/v; pH 7.0) was used. Mobile phase flow rate was maintained at 1 ml/min and effluents were monitored at 210 nm. The sample was injected using a 10 µl fixed loop and the total run time was 10 min.

Calibration curve for levetiracetam

Appropriate aliquots of stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 40,80,120,160,200,240 µg/ml of levetiracetam respectively. The solutions were injected using a 10 µl fixed loop system and chromatograms were recorded. Calibration curve was constructed by plotting average peak area versus concentrations and regression equation was computed for levetiracetam.

Determination of levetiracetam in pharmaceutical formulations

Twenty tablets were weighed and finely powdered. Powder equivalent to 10 mg levetiracetam was accurately weighed and transferred to a 50 ml volumetric flask and 20 ml of diluent was added to the same. The flask was sonnicated for 20 min and volume was made up to the mark with diluent. The above solution was filtered using Whatman filter paper (No.1). Appropriate volume of the aliquot was transferred to a 25 ml volumetric flask and the volume was made up to the mark with mobile phase to obtain 200 µg/ml of levetiracetam. The solution was sonicated for 10 min and injected under above chromatographic conditions and peak area was measured.

Validation:

The calibration curve was obtained at 6 concentration levels of levetiracetam standard solutions. The solutions (10 µl) were injected into liquid chromatographic system (n=6) with chromatographic conditions previously given. The linearity was evaluated by least square regression method. The accuracy of the method was determined by calculating recoveries of levetiracetam by method of standard additions. Known amount of drug were added to a pre-quantified sample solution and the amounts were estimated.

The intra-day and inter-day precision study was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days (first, second and third day) for 3 different concentrations of levetiracetam which represents low, medium and high concentrations in the analytical range. The specificity was estimated by spiking commonly used excipient (starch, talc and magnesium stearate) into a pre weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

The detection limit is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD was the concentration that yielded signal to noise ratio (S/N) 3:1 and LOQ was the concentration that yielded signal to noise ratio (S/N) 10:1.

3.RESULTS AND DISCUSSION

Optimization of mobile phase was carried out by taking different proportions of aqueous and organic phases to obtain rapid, simple assay method for levetiracetam with appropriate run time, asymmetric factor and theoretical plates. Mobile phase consisting of Acetonitrile: 0.01 M potassium dihydrogen phosphate (10:90v/v; pH 7.0) was found to be satisfactory which gave symmetric and sharp peak for levetiracetam at a 1 ml/min flow rate. For quantitative analytical purpose wavelength was set at 210 nm, which provided better reproducibility with minimum interference than the other UV bands. Under the chosen experimental conditions, the liquid chromatogram of levetiracetam showed a single peak of the drug around retention time (Rt) 6.93 min with asymmetry 1.40.

The calibration curve for levetiracetam was obtained by plotting the peak area versus concentration. It was found to be linear in the range of 40-240 µg/ml. Peak area and concentrations were subjected to least square regression analysis to calculate calibration equation and correlation coefficient. The data of the calibration curve are shown in Table 1. The correlation coefficient (r) was found to be 0.999, showing good linearity. Accuracy of the method was examined by performing recovery studies by standard addition method. The recovery of the added standard to the sample was calculated and it was found to be 98.6-100.6%, which indicated good accuracy of the method. Precision studies were carried out at 3 different concentration levels and the results of the intra-day and inter-day studies are reported in terms of RSD Table 1. The LOD value was found to be 0.347 µg/ml which is the concentration that yields signal to noise (S/N) ratio 3:1. The LOQ was 0.715 µg/ml with S/N ratio of 10:1 Table 3.

The proposed liquid chromatographic method was applied to the determination of Levetiracetam in

Tablet formulation A, B and C. The results obtained were satisfactorily accurate and precise as indicated by the good recovery values Table 2.

The method was validated and found to be simple, sensitive, accurate and precise. Statistical analysis proved that method was repeatable and selective for the analysis of levetiracetam without any interference from the excipient.

TABLE 1:Statistical Data For Linearity and Calibration range

Drug	Levetiracetam
Concentration range(µg/ml)	40-240
Slope(m)	11652
Intercept(c)	38.887
Correlation coefficient	0.999
% RSD	0.42

TABLE 2:Results Of HPLC Assay and Recovery Studies

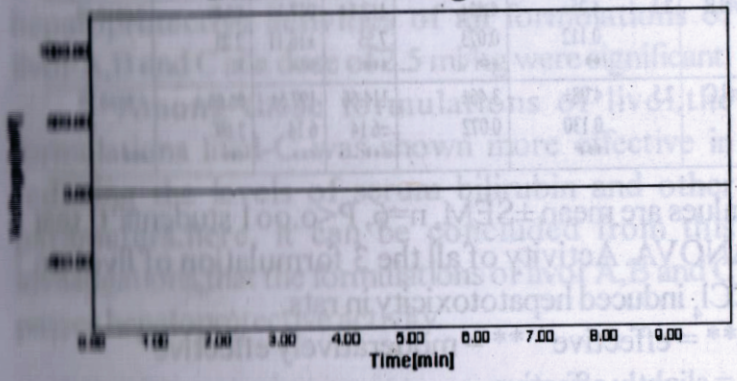
Sample	Amount claim (mg/tablet)	% Found by the proposed method	% Recovery*
A	250	99.2	100.6
B	500	98.8	98.6
C	750	99.6	99.1

*Average of three determinations at three levels

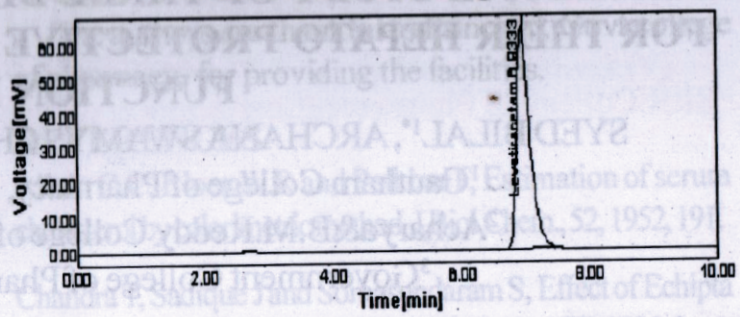
TABLE 3:Validation Summary

Validation parameter	Results
Theoretical plates(N)	7460
Tailing factor	1.4
Retention time(min)	6.93
Area (%)	98.68
LOD (µg/ml)	0.347
LOQ (µg/ml)	0.715

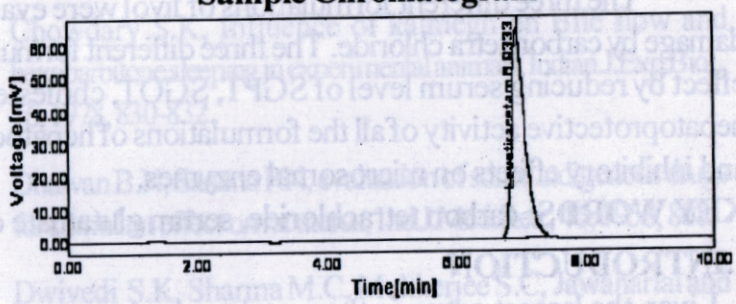
Blank Chromatogram



Standard Chromatogram



Sample Chromatogram



REFERENCES

ICH Harmonized Tripartite guidelines, validation of analytical procedure, methodology, 1996, Nov 6 th, 3.

Isoherranen N, Roeder M, Soback S, Yagen B, Schurig V and Bialer M, J.chromatogr. B, Biomed Sci.Appl., 745(2), 2000, 325.

Jens martens, Lobenhoffer J and Bode-Boger S.M, J.chromatogr.B Analyt.Technol. biomed Life sci., 819(1), 2005, 197.

Physician desk reference, 60 th Edition, 2006, 3307.

Rao B.M, Ravi R, Shyam Sunder Reddy, Siva kumar B,Gopichand S, J.pharm. Biomed Anal.,35(5), 2004, 1017.

Rathnaraj N and Doheny HC, The Drug Monitoring, 18(2), 1996, 154.

Snyder L.R, Practical HPLC and method development, 2nd Ed, 1997, 175.

Tiegong guo, Lisa Oswald, Damodara Rao Mendu and Steven solidin, J. Clin.chim. Acta, 375(1-2), 2007, 115.

www.Rxlist.com/cgi/generic3/keppra.htm.