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Development and validation of an RP-HPLC method for determination of levetiracetam in bulk and pharmaceutical dosage forms

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ABSTRACT

An RP-HPLC analytical method for estimation of Levetiracetam in pharmaceutical dosage forms was developed and validated. A Hypersil ODS C_{18}, 4.6mm×250 mm, 5µm column from Supelco (India), with mobile phase comprised of methanol and ammonium acetate buffer (pH-4) (80:20) with a total run time of 10 min was used and the wavelength of the detector was set at 240 nm. Ritonavir is used as Internal Standard. The retention times were 6.20 min and 5.24 min for Levetiracetam and I.S respectively. The extraction recovery of Levetiracetam from pharmaceutical dosage form (tablets) was >99.8% and the calibration curve was linear (r^2 = 0.999) over Levetiracetam concentrations ranging from 5 to 350µg/ml. The method had an accuracy of >99% and LOD and LOQ of 0.438µg/ml and 1.462µg/ml respectively. The method reported is simple, reliable, precise, accurate and has the capability of being used for determination of Levetiracetam in bulk and pharmaceutical dosage forms.

KEYWORDS

Levetiracetam;
Antiepileptic;
Methanol;
Ammonium acetate buffer;
RP-HPLC.

INTRODUCTION

Levetiracetam (Figure 1) is a novel antiepileptic drug which is structurally and mechanistically dissimilar to other antiepileptic drugs[1]. Its pharmacokinetic profile is linear with respect to dosage, its bioavailability is close to 100%, it undergoes only insignificant hepatic metabolism to inactive metabolites, it does not induce hepatic enzymes and about 91% of the dose is excreted via the renal route[2]. Therefore, it is close to a drug with ideal pharmacokinetic properties. Nevertheless, it is recommended to monitor the plasma concentrations of Levetiracetam to optimize the therapeutic effect, especially in patients with renal impairment, in the elderly where the half-life of the drug is extended[3] and in children, where the half-life is shortened[4]. There are only few papers published reporting therapeutic drug monitoring methods of Levetiracetam. Four of them employed HPLC with UV-detection[5-7,9] and two methods were using GC with NPD-detection[6,8]. These methods were designed for routine therapeutic drug

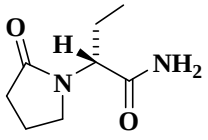

Figure 1: Chemical structure of levetiracetam

monitoring in men. In one of the methods[7], sample preparation with SPE or liquid-liquid extraction is necessary. Pucci et al.[7] evaluated the feasibility of protein precipitation as the only sample preparation step in comparison to SPE. They concluded that protein precipitation is a suitable and fast sample preparation for measuring routine patient samples. J.Martens-Lobenhoffer and S.M.Bode-Boger developed a HPLC-UV method for determination of Levetiracetam in human plasma with minimal sample pretreatment[9]. A Procedure for the Monitoring of Levetiracetam and Zonisamide by HPLC-UV method was also published[10]. Micro emulsion electrokinetic chromatography with UV-detection was utilized in one method[11], but it lacks suitable sensitivity.

Some methods facilitating chiral separation of the S-and R- enantiomer of Levetiracetam, one utilizing GC-MS and the other two HPLC-UV[12,13]. A validated chiral LC method for the enantioselective analysis of Levetiracetam and its enantiomer R-α-ethyl-2-oxo-pyrrolidine acetamide on amylose-based stationary phase[14] and an enantiomeric impurity determination of Levetiracetam using capillary electrochromatography[15] were also published recently. These methods were designed to investigate in dogs the pharmacokinetic and pharmacodynamic properties of the two enantiomers separately. Stability of Levetiracetam drug substances under stressing conditions by LC method was also determined[16].

We here present a new method for the determination of Levetiracetam in bulk and pharmaceutical dosage forms which utilizes a very cheap solvent system on a Hypersil ODS C18 analytical column. This type of method leads to better retention, very sharp and symmetrical peak shapes and exhibits a very good selectivity for Levetiracetam.

MATERIALS AND METHODS

Instrumentation

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC10AT and LC10AT VP series HPLC pumps, with a 20µl Injection of sample loop (manual), and SPD 10A VP UV-Visible Detector. The output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SP1 software. Hypersil ODS C_{18} (46 mm × 25 cm, 5mm) column was used for the separation. The pH of the solution was adjusted by using Digital pH Meter, Model DI 707 (Digisun electronics, Hyderabad, India).

Standards and chemicals

Levetiracetam and Ritonavir were gifts obtained from Aurobindo pharma (Hyderabad, India) and Ranbaxy Laboratories Limited (Mohali, India). Torleve[tablets] (Torrent Pharma) containing 250 mg and 500 mg of Levetiracetam, were purchased from local market. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., USA) water purification system. Methanol of HPLC grade was purchased from Burdick and Jackson (Muskagon, MI, USA), ammonium acetate of A.R. grade and formic acid of A.R. grade were purchased from local suppliers.

A 0.01 M solution of ammonium acetate (pH: 4.0) was prepared by dissolving 0.77g of ammonium acetate in 800 ml water and diluting to 1000 ml with water. The pH was adjusted to 4.0 with formic acid.

Preparation of standard drug solutions

Stock solution of Levetiracetam was prepared by dissolving 25 mg of Levetiracetam in 25 ml of volumetric flask containing 20 ml methanol. The solution was sonicated for about 20 min and then made up to volume with methanol. Daily working standard solutions of Levetiracetam was prepared by suitable dilution of the stock solution with appropriate mobile phase. Similarly stock solution of internal standard was prepared by dissolving 25mg of Ritonavir in 25 ml of methanol.

Chromatographic conditions

The mobile phase used in this study was a mixture of ammonium acetate buffer (0.01M pH 4.0 adjusted with formic acid) and methanol 20:80v/v. The contents of the mobile phase were filtered before use through a 0.45µ membrane and degassed for 15 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 0.5 ml/min. The eluents were monitored at 240nm. The column temperature was maintained ambient through out the experiment. The identification of the separated Levetiracetam and Ritonavir were confirmed by running the chromatograms of the individual compounds under identical conditions.

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TABLE 1: Linearity range of levetiracetam

Concentration (µg/ml)	Ratio(drug/IS)	Statistical analysis
5	0.052	Slope (a) 0.132608
10	0.106	
15	0.158	
20	0.212	Intercept (b) 0.016596
25	0.267	
30	0.316	
35	0.372	
40	0.423	
50	0.534	
60	0.635	
80	0.846	
100	1.068	Correlation coefficient 0.9999
150	1.601	
200	2.114	
250	2.673	
300	3.204	
350	3.721	

TABLE 2 : Amount of Levetiracetam in tablet dosage forms by proposed HPLC method

Formulations Torleve (Torrent)	Labeled amount in mg.	Amount recovered in mg. (n = 6) mean ± S.D	%CV	% Recovery
250 mg Tablet	250	249.5 ± 0.10	± 0.042	99.8
500 mg Tablet	500	499.62 ± 0.67	± 0.136	99.9

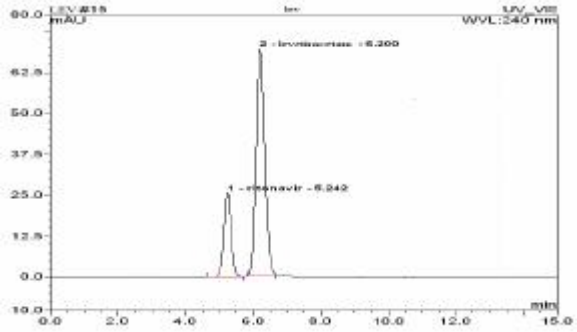

Figure 2: A typical chromatogram of Levetiracetam and I.S (Ritonavir)

Calibration of standards

Calibration standards were prepared by spiking working standard solutions into methanol containing 5 ml volumetric flasks to yield concentrations of 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 80,100, 150, 200, 250, 300 and 350µg /ml. To the above solutions 20µg /ml of Ritonavir (Internal Standard) was added and the final volume was made up to the mark. The represented data was shown in TABLE 1. A 20µl aliquot was injected in to the analytical column. The resultant peak area's of the drug and internal standard was measured. Calibration curve was plotted between peak area ratios of drug and internal standard against concentration of the drug.

Regression equation from 5-350 (g/ml): Y = 0.132608 X + 0.016596, (r^2=0.9999)

Recovery of Levetiracetam in tablets

20 Tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 25 mg of Levetiracetam was extracted with methanol in a 25ml volumetric flask using ultra sonicator. This solution was filtered through Whatmann No 1 filter paper. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined. An aliquot (20µg/ml) of the internal standard was added to the sample solution prior to the dilution. All determinations were carried out in six replicates. The represented data was shown in TABLE 2.

RESULTS

Method validation

Method was validated according to ICH guidelines[17,18]. Specificity and Selectivity of the method was assessed by preparing a drug concentration of 100µg/ml from pure drug stock and commercial sample stock in selected mobile phase and analyzed. The HPLC chromatograms recorded for the drug-matrix (mixture of the drug-excipients) from figure 2 showed almost no peaks within a retention time range of 10 min. The figure 2 shows that Levetiracetam is clearly separated from its internal standard and these are well separated from each other. Thus, the HPLC method presented in this study is Selective for Levetiracetam. The linearity of these methods was evaluated by Linear Regression Analysis, which was calculated by Least Square method and the drug was linear in the concentration range of 5-350µg/ml. Limit of detection was found to be 0.438µg/ml (signal to noise ratio 3) and limit of quantification was found to be 1.462 µg/ml (signal to noise ratio 10). Intra-day precision was studied by six replicate measurements at three concentration levels in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive

TABLE 3: Precision of proposed HPLC method

Sl. no	Concentration taken (µg/ml)	Intra-day Measured Concentration (µg/ml) ± S.D (n = 6)	%CV	Inter-day Measured concentration (µg/ml) ± S.D (n = 6)	% CV
1	20	0.212 ± 0.0014	0.66	0.206±0.0041	0.79
2	60	0.634 ± 0.0015	0.23	0.618±0.0031	0.50
3	250	2.672 ± 0.0020	0.07	2.665±0.0071	0.26

TABLE 4: Accuracy studies

Sl. no.	Amount of standard drug added to 30µg/ml of preanalyzed formulation	Amount of Pure drug recovered Mean ± S.D (µg) (n=6)	% of recovery Mean ± S.D
1	24µg/ml (80%)	23.95 ± 0.06	99.82±0.28
2	30µg/ml (100%)	30.11 ± 0.07	100.38±0.15
3	36µg/ml (120%)	35.99 ± 0.19	99.98±0.53

TABLE 5: System suitability parameters of Levetiracetam

Sl. No.	Parameters	Obtained values
1.	Theoretical plates (N)	2759
2.	Resolution (R)	2.27
3.	Tailing factor (T)	0.914
4.	LOD (µg/ml)	0.438
5.	LOQ (µg/ml)	1.462

days. Accuracy of the method was determined by calculating recovery studies. Statistical evaluation revealed that relative standard deviation of the drug at different concentration levels for six injections was less than 0.79. Precision and accuracy data were shown in TABLES 3 and 4 respectively. For system suitability, six replicates of standard sample were injected and studied the parameters like plate number (N), tailing factor (k), resolution (R) and relative retention time (α), HETP, capacity factor (k^1), plates per meter and peak symmetry of samples. The represented data was shown in TABLE 5. Robustness of the method was done by changing slight variation in the parameters like mobile phase, flow rate and pH of the mobile phase. Present method didn't show any significant change when the critical parameters were modified. The tailing factor for Levetiracetam was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

DISCUSSION

The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic modifier and buffer used in the mobile phase. Retention of Levetiracetam has more dependence on pH of the mobile phase when compared to Ritonavir. The separation of peaks was also dependent on pH of the buffer and the percentage of methanol.

Under the presently prescribed conditions, the recoveries of Levetiracetam were found to be from 99.82 to 100.38 % respectively. This indicates that commonly used excipients in pharmaceutical formulation were not interfering in the proposed method and a very low concentration of buffer (0.01 M ammonium acetate, pH adjusted to 4.0 with formic acid) was used to reduce the tailing of Levetiracetam. This method is very useful for determination of Levetiracetam in pharmaceutical dosage forms, clinical studies and pharmacokinetic studies.

The differences of less than 2.0 % for both intra- and inter-day data reflect the precision of the method. The observation of % C.V less than 2.0 for both intra- and inter-day measurements also indicates high degree of precision. In the present method, a Hypersil ODS C18 column has been used and the buffer pH in the mobile is 4.0, which is within the limits (pH 2-8) specified by the manufacturers. In the present method, we have established a linearity range of 5-350µg /ml; this linearity range covers all the strengths of Levetiracetam. Hence this method can be applied for quantifying the low levels of Levetiracetam in pharmaceutical dosage forms and other pharmacokinetic studies.

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