Research Journal of Recent Sciences _____ ISSN 2277-2502
Vol. **2(5),** 68-70, May **(2013)** *Res.J.Recent Sci.*

Short Communication

Estimation of Levetiracetam in Bulk and Pharmaceutical Dosage Form with a Newly Developed and Validated RP-HPLC Method

Muhammad Usman[1], Hafsa Bibi[2], Muhammad Tariq Khalil[1], Altaf Hussain[3], Aisha Siddiqua[2] and Javeid Iqbal[4]
[1]Mohi Ud Din Islamic Institute of Pharmaceutical sciences, Mirpur, Azad Jammu and Kashmir, PAKISTAN
[2]Department of Chemistry, Gomal University, Dera Ismail Khan, PAKISTAN
[3]PIMS, Islamabad, PAKISTAN
[4]Faculty of Pharmacy; Hamdard University, Karachi, PAKISTAN

Available online at: www.isca.in
Received 13th December 2012, revised 3rd January 2013, accepted 2nd February 2012

Abstract

A reversed-phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of Levetiracetam in bulk and tablets dosage forms. The separation was achieved on C_{18} analytical column (250 mm × 4.6 mm i.d., 5.0 µm) using acetonitrile and distilled water in the ratio 80:20 v/v as mobile phase and at a flow rate of 1.0 mL/min. Detection was carried out using a UV detector at 210nm. The total chromatographic analysis time per sample was about 5.0min with Levetiracetam eluting at retention time of about 3.5min. The method is accurate (99.51% - 100.45%), and the standard curve was linear over the concentration range of 25-150µg/mL with R^2 close to one (0.999) and Y-intercept of 0.022. The limit of detection (LOD) and limit of Quantitation (LOQ) obtained for Levetiracetam were 0.01µg/mL and 0.09µg/mL, respectively. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the determination of Levetiracetam in tablets dosage form.

KeyWords: RP-HPLC, accuracy, precision, tablets, levetiracetam.

Introduction

Levetiracetam is a FDA approved antiepileptic drug and is used in clinical conditions partial seizures and myoclonic jerks[1]. The IUPAC or systemic name of levetiracetam is (*S*)-2-(2-oxopyrrolidin-1-yl) butanamide[2]. The bioavailability of levertiracetam after oral administration, is almost equal to 100%. The biotransformation occurs by the enzymetic hydrolysis of acetamide group. The metabolized drug is excreted through urine[3].

There are various methods in the literature for the qualitative and quantitative analysis of the Levetiracetam in the bulk and the pharmaceutical dosage forms. The method was developed and validated under the light of International Conference on Harmonization (ICH) guidelines. And for the statistical evaluation of results, standards guidelines were followed. Hence, our aim was to establish an easy and convenient high pressure liquid chromatography (HPLC) technique, which not only useful for researcher but also for the analysts working in the pharmaceutical quality control labs.

Material and Methods

The reference standards of Levetiracetam (99.87% purity) was received from Global Pharmaceuticals Pvt. Ltd, Islamabad, as donation taster. The EPPRA® tablets (Global Pharmaceuticals, Islamabad) declaring film coated tablets equivalent to 250mg were obtained from the habitat pharmaceutical marketplace. The acetonitrile used in the study was of HPLC grade and was obtained from the Merck's local distributor.

Apparatus and Chromatographic Conditions: HPLC system *(Shimadzu)*: Isocratic with LC20AD pump andSPD-20A UV-visible detector Column (*thermolab)*: 250 x 4.6mm (5µm internal diameter) with ODS-3 packaging.

λmax : 210nm on ambient temperature, Run time: 5 minutes, Flow rate: 1ml/minute

Preparation of Mobile Phase: The mobile phase was prepared by mixing distilled water and acetonitrile in 20:80 (v/v) ratios. The final mobile phase was then filtered by passing through 0.5µm membrane filter and degassed before use.

Preparation of Standard Solution: Standard solution was prepared by dissolving Levetiracetam reference quivalent to 100mg of Levetiracetam in 100 mL of mobile phase (final concentration, 1 mg/mL). This solution was filtered through 0.2µm membrane filter and 20 µL of this solution was injected for HPLC analysis.

Tablets Sample Preparation: For the assay of Levetiracetam, 20 tablets were weighed; their contents were crushed into fine powder and mixed thoroughly. Amount of tablets powder equivalent to 100mg of Levetiracetam is dissolved in 100 mL of mobile phase (final concentration, 1 mg/mL). This solution was filtered through 0.2µm membrane filter and 20 µL of this solution was injected for HPLC analysis.

Method Validation: The method was validated for the parameters like specificity, range and linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision. In addition, system suitability parameters were also calculated[4,5].

Results and Discussion

First of all system suitability was evaluated. The table 1 shows the result for these parameters. The column efficiency was much better for analysis i.e. ≥2000. The tailing factor was also within range i.e. ≥1.2. Moreover, the calculated relative standard deviation for the retention time and peak area (mean of 6 replicates) also within acceptance criteria. Depending on all these information, it reflects that the proposed method will be suitable for routine analysis.

Table-1
System suitability

S. No	Parameters	Levetiracetam
1	Retention time (min)	3.5
2	Theoretical Plates	2651
3	Tailing factor	0.727
4	RSD of peak area (n=6)	0.81
5	RSD of retention time (n=6)	0.76

For accuracy of the method, solution of Levetiracetam with different concentration (25, 50, 75, 100, 125, and 150%) was prepared and analyzed via proposed method. During this step, six samples of each concentration were prepared and their mean was used for further calculations. The results are shown in the table 2, that show that the recovery of Levetiracetam from the prepared samples ranges from 98.63% to 101.23% i.e. within ±1% range. Moreover, the RSD (relative standard deviation) also lies within acceptance range i.e. ≤2.0.

In order to check the precision of the method, repeatability of method was analyzed by replicate analysis (n=6). The results are shown in the table 3 which indicates that the proposed method is good with high precision. Moreover, the low RSD values indicate the high degree of correctness of method. Similarly, for reproducibility was checked by replicate analysis (n=18) of samples over 3 consecutive days. From results (given in table 3), the low calculated RSD reflects that the method has a good inter-day reproducibility.

The linearity of the method was checked by preparing different strengths solution of Levetiracetam from 25% to 150%. From the observation and calculation (given in table 4), it is cleared that the correlation coefficient (R^2) equal to unity and comes under t he acceptance criteria ($R^2 \geq 0.999$). Moreover, the calculated Y-intercept is 0.022 which is also less than ±2%. Therefore, depending upon calculated values of R^2 and Y-intercept, the developed method should be considered having a high degree of linearity.

Calibration curves were constructed in a very low concentration region (0.05 to 1.0% of the target concentration) of Levetiracetam for the calculation of the limit of detection (LOD) and the limit of quantification (LOQ). The LOD and LOQ obtained for Levetiracetam were 0.010µg/mL and 0.09µg/mL, respectively".

The proposed method was also applied to the pharmaceutical dosage (tablets in this case) form of the Levetiracetam. For this purpose 3 batches were selected and 6 replicates of each batch were analyzed by the HPLC, from the results (table 5), it was observed that the obtained results are in good agreement with the claimed amount of Levetiracetam by the manufacturer.

Table-2
Accuracy of Method

S.NO	Concentration Level (%age)					
	25	**50**	**75**	**100**	**125**	**150**
1	99.81	100.31	101.11	99.43	99.71	100.23
2	100.22	99.25	100.88	100.78	99.62	100.31
3	101.23	98.91	100.91	100.32	101.00	99.70
4	100.54	99.65	99.88	99.56	100.44	98.63
5	99.45	100.42	100.27	99.29	100.23	99.45
6	99.66	100.26	100.33	99.84	99.24	100.28
Mean	100.151	99.8	100.563	99.87	100.04	99.766
%RSD	0.658	0.628	0.474	0.575	0.639	0.658

Table-3
Inter day and intraday precision of the method

S.No:	Recovery (%age)		
	Day 1	**Day 2**	**Day 3**
1	100.21	99.77	100.41
2	99.24	99.28	100.69
3	100.45	100.10	100.38
4	99.78	100.81	99.42
5	100.58	99.61	99.56
6	99.21	100.53	100.29
Mean	99.917	100.167	100.125

Table-4
Linearity of the Method

S.No:	Drug Dissolved	Drug Recovered
1	25mg	24.56mg
2	50mg	50.13mg
3	75mg	75.22mg
4	100mg	100.39mg
5	125mg	124.78mg
6	150mg	149.71mg
Correlation Coefficient: 0.999		
Y-intercept: 0.022		
Regression Equation: 0.022+0.999X		

Table-5
Assay Results of 250mg Levetiracetam Tablets (Lumark)

B. No.	Drug Recovered (mg)±SD
1	248.98±0.671
2	249.42±0.551
3	250.13±0.643

Note: n=6; SD=Standard Deviation

Conclusion

A simple isocratic RP-HPLC method has been developed for the determination of Levetiracetam in bulk and tablets dosage form, using a UV detector. The method was validated for accuracy, precision, specificity and linearity. The method has a relatively short run time (about 5min) that allows quantifying a large number of samples in routine and quality control analysis of tablets.

References

1. Tripathi K.D., Essential of Medical Pharmacology, 6th ed., 410 **(2010)**

2. The Merck Index, 13th Edn., Merck and Co., Inc., Whitehouse Station, NJ, 978 **(2001)**

3. Farooq M.U., Bhatt A., Majid A., Gupta R., Khasnis A., Kassab M.Y., Levetiracetam for managing neurologic and psychiatric disorders, *Am J Health Syst Pharm,* (**66**)6, 541–61 **(2009)**

4. ICH, Q2 (A), Validation of analytical procedures: text and methodology International Conference on Harmonization. Geneva, 1- 13 **(2005)**

5. International Conference on Harmonization, Guideline on Validation of Analytical Procedure-Methodology, Geneva, Switzerland **(1996)**