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Analytical method development and validation for the determination of Levetiracetam in pharmaceutical formulations by using RP-HPLC

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ABSTRACT

A simple, reproducible and efficient reversed phase high performance liquid chromatography (RP-HPLC) method has been developed for estimation of a recently approved anti epileptic drug, Levetiracetam in raw material and its pharmaceutical formulations. Separation was done by using mobile phase consisting of diphasic sodium phosphate buffer and acetonitrile in a ratio of 80:20. The separations were carried out on an Inertsil C_{18} column (250x 4.6mm; 5µm) at a flow rate of 1.5 ml/min. The injection volume was 10 µl and the peaks were detected at 205nm. The linear dynamic response was found to be in the concentration range of 80 µg -130 µg/ml and coefficient of correlation was found to be 0.9994. The percentage recovery of levetiracetam was found to be 98.37%. The proposed method was found to be simple, fast, accurate, precise and reproducible and could be used for routine quality control analysis of levetiracetam in bulk and pharmaceutical dosage forms.

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

INTRODUCTION

Levetiracetam (Fig-1) is a novel antiepileptic agent; with a chemical name (S)-(2)-(2-oxypyrrolidin-yl) butamide[1]. It is used as an adjunctive therapy in the treatment of partial seizures. Levetiracetam can prevent myoclonic jerks and generalizes epileptiform activity in patients with photosensitive epilepsy[2].

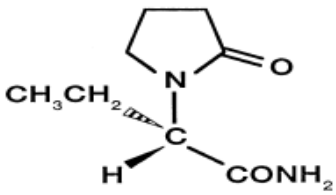


Figure 1: Structure of Levetiracetam

Various HPLC[3-8] and LC-MS[9-11] methods have been reported for the determination of levetiracetam in pure and pharmaceutical dosage forms. In this present investigation an attempt has been made to develop an accurate, precise, simple and sensitive reversed phase high performance liquid chromatography method for the estimation of levetiracetam in bulk and in pharmaceutical dosage form.

MATERIALS AND METHODS

Experimental

Chemicals and reagents

Acetonitrile of HPLC grade was purchased from E.Merck (India) Ltd., Mumbai. Dibasic sodium phosphate and orthophosphoric acid of AR grade were obtained from Qualigens Fine Chemicals Ltd., Mumbai. Levetiracetam was a gift sample by The Madras Pharmaceuticals, Chennai. The commercially available Levetiracetam oral solution was procured from the local market.

Instrumentation and chromatographic conditions

The chromatographic separation was carried out on HPLC system (Shimadzu Co, Tokyo, Japan) with UV- Visible dual absorbance detector (PDA), Inertsil C_{18} column (250 x 4.6mn; 5µm). The mobile phase consisting of dibasic sodium phosphate buffer (pH 3.5 adjusted with orthophosphoric acid) and acetonitrile were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 80:20 v/v was pumped into the column at a flow rate of 1.5 ml/min. The detection was monitored at 205 nm. The volume of injection loop was 10 µl prior to the injection of the drug solution; the column was equilibrated for at least 30 min. with the mobile phase following through the system. The column and the HPLC system were kept in ambient temperature (25º C).

Preparation of stock solution

50 mg of Levetiracetam was weighed and transferred carefully in 25 ml volumetric flask. About 20 ml of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase. 5 ml of above solution was diluted to 100 ml with mobile phase. (100 µg/ml)

Analysis of oral solution formulation

Accurately weighed a portion of an oral solution equivalent to 200 mg of Levetiracetamin a clean and dry 100 ml volumetric flask. 70 ml of mobile phase was added, sonicated to dissolve for 5 to 10 min. with intermittent shaking and make up the volume with mobile phase and filtered through 0.45µ membrane filter. (100 µg/ml)

Amount of Levetracetam in oral solution:

$$= \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times \frac{\text{Potency}}{100} \times \text{Wt/ml}$$

RESULTS AND DISCUSSION

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines[12].

System Suitability

It is essential for the assurance of the quality performance of chromatographic system. Five injections of standard drug solution were given to the system. The mean area. Standard deviation and %RSD were calculated and mentioned in Table 1. The system suitability parameters such as retention time, tailing factor, number of theoretical plate and peak area response were also be calculated and mentioned in Table 2. It was observed that all the values are with in the limits.

Table 1: System suitability for Levetiracetam

S.No.	Standard	Concentration (µg/ml)	Area
1.	Standard -1	100	1350758
2.	Standard -2	100	1350785
3.	Standard -3	100	1351115
4.	Standard -4	100	1352178
5.	Standard -5	100	1351443
		Mean	1351256
		Standard deviation	586.255
		RSD in %	0.034

Table 2: System suitability parameters for Levetiracetam

S.No.	System suitability parameters	Levetiracetam
1.	Retention time	2.77 min
2.	Tailing factor	1.327
3.	Number of theoretical plate	5240.143
4.	Peak area response	1350758

Table 3: System suitability parameters for Levetiracetam

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	1352497
4.	Sample	1	1394776

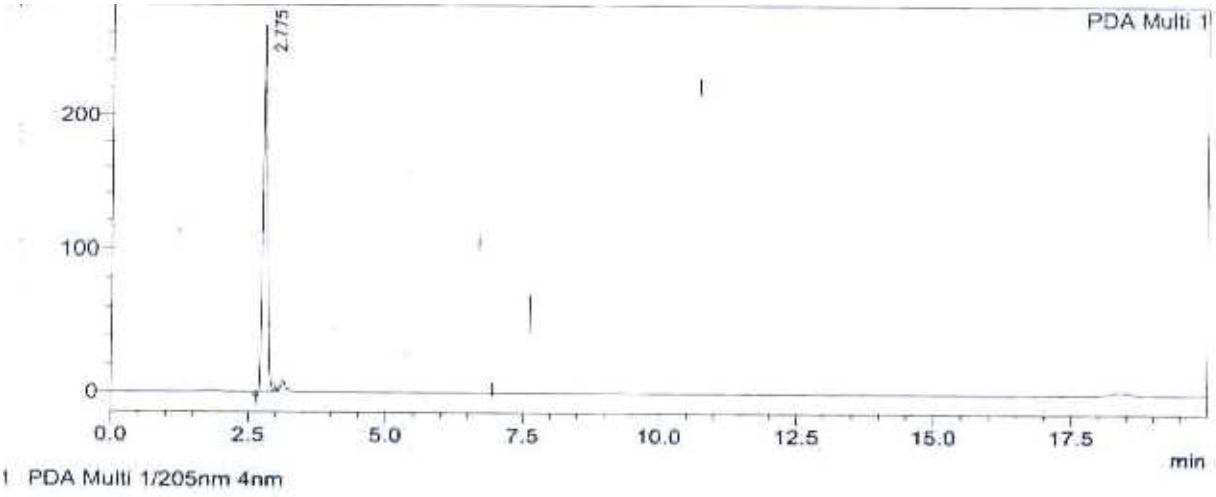


Figure 2: Typical chromatogram of Levetiracetam oral solution

Specificity

The specificity of the HPLC method is illustrated in Fig. 2 where complete separation of Levetiracetam was noticed in presence of tablet excipients. In addition there was no any interference at the retention time of in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte.

Limit of Detection (LOD)

LOD was determined by calibration curve method. Different concentration levels were prepared & analysed. LOD data was shown in Table 4. The lower limit of detection was calculated by using the formula: LOD = 3.3 x σ / S; where σ – standard deviation of y-intercepts of regression line, S – slope of the calibration curve. The LOD was found to be 0.015 µg/ml. The results demonstrated that the method was highly sensitive.

Table 4: LOD for Levetiracetam

S.No	Concentration (µg/ml)	Standard weight (mg)	Standard Area	Mean	Standard deviation	RSD (%)
1.	0.6	201.1	8371	8462.0	123.0488	1.4541
			8413			
			8602			
2.	0.8		11132	11231.67	91.5442	0.8151
			11251			
			11312			
3.	1.0		13918	13804.33	149.4869	1.0829
			13635			
			13860			
4.	1.2		16404	16442.33	105.8411	0.6437
			16361			
			16562			

Table 5: LOQ for Levetiracetam

S.No	Sample	Weight taken	Concentration (µg/ml)	Area	% of Assay
1.	Standard	201.1	4	55365	---
2.	Standard		4	55477	---
3.	Standard		4	55188	---
4.	Standard		4	55165	---
5.	Standard		4	55307	---
6.	Standard		4	55185	---
7.	Sample - 1	2521.6	4	57811	102.53
8.	Sample - 2	2490.2	4	58661	105.35
9.	Sample - 3	2486.4	4	57703	103.79
10.	Sample – 4	2533.3	4	58272	102.87
11.	Sample - 5	2512.4	4	57622	102.57
12.	Sample - 6	2504.8	4	57175	102.08
				Mean	103.198
				Standard deviation	1.198
				RSD in %	1.16

Limit of Quantitation (LOD)

LOQ was determined by calibration curve method. Different concentration levels were prepared & analysed. LOQ data was shown in Table 5. The lower limit of quantitation was calculated by

using the formula: LOQ = 10 x σ / S; where σ – standard deviation of y-intercepts of regression line, S – slope of the calibration curve. The LOQ was found to be 0.047 µg/ml. The results demonstrated that the method was highly sensitive.

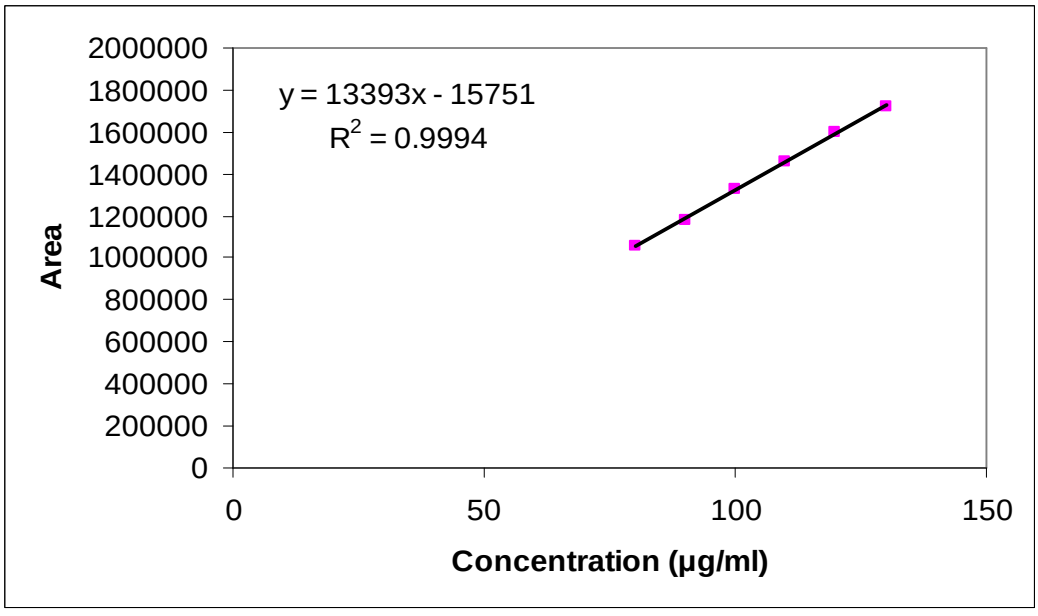
Linearity of Response

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of analyte in the samples within a given range. The Linearity of this method was determined at six concentration levels from 80µg/ml – 130 µg/ml (Table 6). The plot of peak area of each sample against respective concentration of Levetiracetam was found to be linear (Figure 3) in the range of 80-130µg/ml. Beer's law was found to be obeyed over this concentration range. The linearity was evaluated by linear regression analysis using least square method. The regression equation was found to be Y= 13393 x - 15751 and correlation coefficient of the standard curve was found to be 0.9994. It observed that correlation coefficient and regression analysis are with in the limits.

Table 6: Linearity of response for Levetiracetam

S.No	Linearity Level	Concentration (µg/ml)	Volume of stock solution (ml)	Volume made up to (ml)	Area	Mean
1.	Linearity -1	80	4.0	100	1055381 1055190	1055285.5
2.	Linearity -2	90	4.5	100	1182957 1183616	1183286.5
3.	Linearity -3	100	5.0	100	1328782 1328486	1328634.0
4.	Linearity -4	110	5.5	100	1459421 1460512	1459966.5
5.	Linearity -5	120	6.0	100	1598022 1598113	1598067.5
6.	Linearity -6	130	6.5	100	1717930 1717345	1717637.5

Figure 3: Linearity of Levetiracetam



Accuracy

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standard Levetiracetam was added to pre-analysed samples at a level from 80% up to 120% and then subjected to the proposed HPLC method. The results of recovery studies were shown in Table 7. It was observed that the mean percentage recovery of Levetiracetam was 98.3667 which demonstrated that the method was highly accurate.

Table 7: Accuracy for Levetiracetam

S. No.	% Recovery/ concentration	Placebo weight (mg)	Standard Weight (mg)	Standard Area	Synthetic mixture Area	Amount recovered (mg)	Recovered (%)
1.	Standard	---	201.1	1356987	---	---	---
2.	80	86.5		---	1068034	78.71	98.39
3.	80	85.7		---	1067419	78.66	98.33
4.	80	90.2		---	1068129	78.71	98.39
5.	100	92.4		---	1337509	98.56	98.56
6.	100	86.4		---	1333522	98.27	98.27
7.	100	86.1		---	1332196	98.17	98.17
8.	120	84.5		---	1601458	118.02	98.35
9.	120	90.5		---	1601623	118.03	98.36
10	120	92.7		---	1603632	118.18	98.48
Mean							98.3667
Standard deviation							0.1123
RSD in %							0.114

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

Reproducibility

Examines the precision between laboratories and is often determined in collaborative studies. Reproducibility data for Levetiracetam was shown in Table 8. This indicated that method was highly precise.

Table 8: Precision - Reproducibility for Levetiracetam

S.No.	Sample Name	Concentration (µg/ml)	Area
1.	Standard -1	100	1360486
2.	Standard -2	100	1361209
3.	Standard -3	100	1360225
4.	Standard -4	100	1361221
5.	Standard -5	100	1360979
Mean			1360824
Standard deviation			448.03
RSD in %			0.0329

Repeatability

Repeatability is the precision of a method under the same operating conditions over a short period of time. One aspect of this is instrumental precision. A second aspect is sometimes termed intra-assay precision and involves multiple measurements of the same sample by the same analyst under the same conditions. Repeatability data for Levetiracetam was shown in

Table 9. This indicated that method was highly precise.

Table 9: Precision - Repeatability for Levetiracetam

S.No.	Sample Name	Wt. taken (mg)	No. of readings	Area	Assay (%)
1.	Standard -1	201.1	2	1361443	---
2.	Sample -1	2521.6	1	1393130	100.33
3.	Sample -2	2490.2	1	1378514	100.53
4.	Sample -3	2486.4	1	1368950	99.98
5.	Sample -4	2533.3	1	1389867	99.63
6.	Sample -5	2512.4	1	1384639	100.08
7.	Sample -6	2504.8	1	1380461	100.08
Mean					100.11
Standard deviation					0.3078
RSD in %					0.3074

Robustness

Measure of method's capacity to remain unaffected by small, but deliberate variations in method.

Change in wave length **(± 2.0 nm)**

Three sample preparations will be analyzed as per the methodology at two different wavelengths i.e. 203 nm and 207 nm. The robustness data by changing wavelength for Levetiracetam was shown in Table 10. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

Table 10: Robustness - Change of wave length for Levetiracetam

S.No.	Sample Name	Wt. taken (mg)	Area		Assay (%)	
			at 203 nm	at 207 nm	at 203 nm	at 207 nm
1.	Standard preparation	201.1	1567086	1112902	-----	----
			1566401	1113120		
2.	Sample -1	2521.6	1599458	1136627	100.09	100.12
3.	Sample -2	2490.2	1583142	1124987	100.32	100.35
4.	Sample -3	2486.4	1571359	1116084	99.73	99.71
Mean					100.047	100.06
Standard deviation					0.2973	0.32419
RSD in %					0.2972	0.324

Table 11: Robustness -Change of Temperature for Levetiracetam

S.No.	Sample Name	Wt. taken (mg)	Area		Assay (%)	
			at 28º C	at 32º C	at 28º C	at 32º C
1.	Standard preparation	201.1	1366466	1375120	-----	-----
			1366931	1373656		
2.	Sample -1	2521.6	1395880	1392143	100.14	99.31
3.	Sample -2	2490.2	1382504	1378325	100.43	99.56
4.	Sample -3	2486.4	1371955	1365946	99.81	98.82
Mean					100.127	99.23
Standard deviation					0.3102	0.3764
RSD in %					0.3098	0.3793

Change of Temperature **(± 2^{o}C)**

Three sample preparations will be analyzed as per the methodology at two different temperature i.e. 28^{o}C and 32^{o}C. The robustness data by changing temperature for Levetiracetam was shown

in Table 11. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

CONCLUSION

In this present study an attempt has been made to develop Reversed Phase – High Performance Liquid Chromatography (RP-HPLC) method for the determination of levetiracetam in pure sample and solution dosage form. The results obtained were reproducible and reliable. The validity and precision of the methods were evident from the statistical and analytical parameters obtained. Therefore, it is concluded that the proposed RP-HPLC method was found to be simple, rapid, sensitive, precise, economical and accurate. Hence, this method can easily and conveniently adopt for routine quality control analysis of levetiracetam in pure sample and its pharmaceutical formulations.

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REFERENCES

[1] Martindale, *The Complete drug reference*, **2005**, 34, 366.
[2] F. Lancelin, E. Franchon, L. Kraoul, I. Garciau, S. Brovedani, K. Tabaouti, E. landre, F. Chassoux, P. Paubel and M.L. Piketty, *Ther Drug Monit*, **2007**, 29(5), 576-583.
[3] C. Manuela, M. Susan, A. Fiorenzo, R. Roberto and B. Agostino, *J chromatogr B.*, **2008**, 873(1), 129.
[4] N. Appala Raja, J. Venkateswara Rao, K. Vanitha Prakash, K. Mukkanti and K. Srinivasu, *E-Journal of Chemistry*, **2008**, 5(S2), 1098.
[5] J. Valarmathy, L. Samueljoshua, G. Rathinavel, C. Selvin Thanija and T. Sivakumar, *Research J Pharm and Tech*., **2008**, 1(3), 395.
[6] J. Marten Lobenhoffer and S.M. Bode Boger, *J Chromatogr B.*, **2005**, 815, 197.
[7] N. Ratnaraj, C. Doheny Helen and N. Patsalos Philip, *Ther Drug Monit*, **1996**, 18(2), 154.
[8] A.C. Vermeij and P.M. Edelbroek, J Chromatogr B., **1994**, 662, 134.
[9] M. Kamal Matar, *J Pharm Biomed Anal*., **2008**, 48(3), 822.
[10] G. Saravanan, G. Jhothy, Y. Suresh, A. Annerao, M. Ramakrishna, M. Yogeshwar Reddy and B. Ravibabu, *Chromatographia*, **2008**, 67, 173.
[11] Tiedong Guo, M. Lisa Oswald, M. Damodara Rao and J. Steven Soldin, *Clinica Chimica Acta*, **2007**, 375, 115.
[12] ICH guidelines, *analytical method validation* (Q3). Geneva, July **2000**.