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Original Article

Stability Indicating RP-HPLC Method for Quantitative Estimation of Levetiracetam and its Impurities in Pharmaceutical Dosage Form

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ABSTRACT

Objectives: A simple, precise, accurate, robust and selective stability-indicating reverse phase high performance liquid chromatographic method for the separation and quantification of Levetiracetam and its impurities in Levetiracetam liquid dosage formulations. **Materials and Methods:** The analysis of improved RP-HPLC method for the separation and quantification of Levetiracetam and its impurities are described. Samples are analysed by means of reverse phase (RP-HPLC) using an Inertsil ODS-3V, 150 x 4.6 mm, 3µm and the mobile phase consists of two channels A and B. Channel-A: pH 5.50 phosphate buffer : acetonitrile (950:50 v/v) and channel-B: acetonitrile: water (90:10 v/v). The flow rate is 1.0 ml/min. The column temperature was maintained at 40°C and sample temperature was maintained at 25°C, injection volume 10µL and wavelength fixed at 205 nm. **Results:** For selectivity, the chromatograms were recorded for standard and sample solutions of Levetiracetam and its related substances. Selectivity studies reveal that the peak is well separated from each other. Therefore the method is selective for the determination of related substances in Levetiracetam. There is no interference of diluent and placebo at Levetiracetam and impurities peaks. The elution order and the retention times of impurities and Levetiracetam obtained from individual standard preparations and mixed standard preparations are comparable. The limit of detection (LOD) and limit of quantitation (LOQ) for Levetiracetam standard 0.0023% and 0.0070%, impurity-A 0.0049% and 0.0147%, impurity-C 0.0024% and 0.0074% and Levetiracetam RC-A 0.0091% and 0.0277% respectively. The linearity results for Levetiracetam and all the impurities in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.Calibration curve was plotted and correlation co-efficient for Levetiracetam and its impurities found to be 1.000, 0.9999, 1.000 and 0.9994 respectively. The accuracy studies were shown as %recovery for Levetiracetam and its impurities at specification level. The limit of % recovered shown is in the range of 80 and 120% and the results obtained were found to be within the limits. Hence the method was found to be accurate. For precision studies six replicate injections were performed. %RSD was determined from the peak areas of Levetiracetam and its impurities. The acceptance limit should be not more than 10, and the results were found to be within the acceptance limits. **Conclusion:** The developed LC method was validated with respect to specificity, precision, linearity, ruggedness and robustness. Validation study compared as per ICH guideline.

Keywords: Levetiracetam, Determination of related substances, Forced degradation, Liquid chromatography.

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INTRODUCTION

Levetiracetam is an antiepileptic drug used in treatment of epilepsy, partial onset, myoclonic or tonic-clonic seizures. It is S-enantiomer of Levetiracetam. Its chemical name is (S)-2-(2-oxopyrrolidin-1-yl) butanamide. It acts by binding to SV2A (synaptic vesicle glycoprotein 2A) and inhibits presynaptic calcium channels reducing neurotransmitter release and acting as a neuromodulator.[1-3] Its empirical formula is $C_8H_{14}N_2O_2$, and molecular weight 170.212 g/mol. Levetiracetam is a white to off-white free-flowing crystalline powder. Levetiracetam is very soluble in water and freely soluble in chloroform, methanol and ethanol; polymorphism has not been observed in induction studies. Its structural formula is shown in Figure 1.

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The extensive literature survey revealed few methods are developed to estimate the drug Levetiracetam in raw material, tablets and in biological fluids using UV,[4-6] RP-HPLC,[7-17] LC-MS,[18-20] Capillary electrophoresis,[21] UPLC,[22,23] Gas Chromatography[24,25] and HPTLC.[26] However, there is no stability indicating method developed yet for the estimation of Levetiracetam and its impurities by RP-HPLC in liquid dosage forms. The objective of the present work is to develop a stability indicating HPLC method and validated as per ICH[27] and Q2(R1) validation guidelines.

EXPERIMENTAL

Chemicals and reagents

Potassium dihydrogen orthophosphate, potassium hydroxide, Hydrochloric acid, Sodium hydroxide and Hydrogen peroxide purchased from Merck, Mumbai, India. Acetonitrile and Milli-Q water HPLC grade procured from Merck, India.

Preparation of buffer

Weighed accurately 2.7 g of Potassium dihydrogen orthophosphate and transferred into a 1000 mL of water and mixed well. And adjusted the pH to 5.5 with 2% aqueous potassium hydroxide.

Preparation of mobile phase-A

Prepared a mixture of 50 mL of Acetonitrile and 950 mL of buffer in the ratio of 50:950 (%v/v). Filtered through 0.45 membrane filtered and sonicated to degas.

Preparation of mobile phase-B

Use 100% Acetonitrile as mobile phase-B.

Preparation of diluent

Mobile phase-A was used as diluent.

Preparation of standard solution

Accurately weigh and transfer about 5.0 mg of Levetiracetam standard into 10 mL volumetric flask, add 5 mL of diluent, sonicate for 5 min to dissolve, make up to the mark with diluent and mix well. Transfer 1.0 mL of above solution into 100 mL volumetric flask, make up to mark with diluent and mix well.

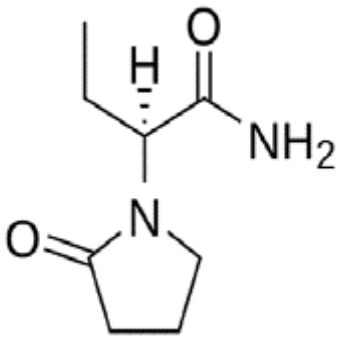

Figure 1: Chemical structure of Levetiracetam.

Preparation of sensitivity solution

Transferred 1.0 mL of standard solution into 10 mL volumetric flask, made up to mark with diluent and mixed well.

Preparation of placebo solution

Transferred 1.0 mL of placebo solution into a 20 mL of volumetric flask, diluted to volume with diluent and mixed well.

Preparation of sample solution

Transferred 1.0 mL of sample solution into a 20 mL of volumetric flask, diluted to volume with diluent and mixed well.

Preparation of impurity-A stock solution

Accurately weighed and transferred 3.091 mg of Levetiracetam impurity-A in to 10 mL volumetric flask. Dissolved the contents and made up to the mark with diluent and mixed well.

Preparation of impurity-C stock solution

Accurately weighed and transferred 3.197 mg of Levetiracetam impurity-C in to 20 mL volumetric flask. Dissolved the contents and made up to the mark with diluent and mixed well.

Preparation of Levetiracetam RC-A stock solution

Accurately weighed and transferred 3.080 mg of Levetiracetam RC-A in to 20 mL volumetric flask. Dissolved the contents and made up to the mark with diluent and mixed well.

Preparation of spiked sample solution

Transferred 1 mL of sample solution into 20 mL of volumetric flask containing 5 mL diluent and then added 1.0 mL of impurity-A stock solution, 0.17 mL of impurity-C stock solution and 0.34 mL of Levetiracetam RC-A stock solution made up to the volume with diluent and mixed well.

Chromatographic conditions

Analysis was carried out on Waters 2489 UV-visible detector/2695 Separation Module, equipped with Empower[3] software. Inertsil ODS-3V (150x4.6mm, 3µm) column was used as stationary phase. The mobile phase consists of two channels A and B. channel-A: pH 5.50 phosphate buffer: acetonitrile (950:50 v/v) and channel-B: acetonitrile: water (90:10 v/v). The flow rate is 1.0 mL/min. The column temperature was maintained at 40°C and sample temperature was maintained at 25°C, injection volume 10µL and wavelength fixed at 205 nm respectively.

Table 1: Impurity interference data (Specificity results).

Peak Name	Retention Time	Blank	Placebo
Blank	ND	NA	NA
Placebo	ND	NA	NA
Impurity-A	5.840	No	No
Impurity-C	3.816	No	No
Levetiracetam RC-A	12.443	No	No
Levetiracetam	9.403	No	No

MATERIALS AND METHODS

Method Development

Method optimization parameters

An understanding of the nature of API (functionality, acidity, or basicity), the synthetic process, related impurities, the possible degradation pathways and their degradation products are needed for successful method development in reverse-phase HPLC. In addition, successful method development should result a robust, simple and time efficient method that is capable of being utilized in manufacturing setting.

Selection of wavelength

The sensitivity of the HPLC method depends upon the selection of detection wavelength. An ideal wavelength is one that gives good response for related substances and the drugs to be detected. The wavelength for measurement was selected as 205 nm from the absorption spectrum.

Selection of stationary phase

Proper selection of the stationary phase depends up on the nature of the sample and chemical profile. The drug selected for the present study was polar compound and could be separated either by normal phase chromatography or reverse phase chromatography. From literature survey, it was found that different C_{18} columns could be appropriately used for the separation of related substances for Levetiracetam.

Selection of mobile phase

Different mobile phase and stationary phases were employed to develop a suitable LC method for the quantitative determination of impurities in Levetiracetam. Different mobile phase composition were tried to get good peak shapes and selectivity for the impurities present in Levetiracetam.

Poor peak shape and resolution was observed when Hypersil BDS C_{18} (150mm x 4.6mm, 3µ) and gradient mobile phase programmed of mobile phase: A pH 2.80 phosphate buffer and mobile phase: B Acetonitrile. There was no proper resolution of impurities and analyte peak and efficiency of the peak is also not achieved and peak interferences are present.

In second attempt made using Inertsil ODS-3V, 150 x 4.6 mm, 3µm column, and gradient mobile phase programmed of mobile phase: A pH 5.50 phosphate Buffer:Acetonitrile and mobile phase: B Acetonitrile: water. The resolution of both drug and impurities was achieved. These chromatographic conditions were selected for validation studies.

METHOD VALIDATION RESULTS

Specificity

Specificity was demonstrated by injected blank solution, placebo solution, standard solution, sample solution, spiked sample and individual impurities and analyzed as per the test method. The observations are tabulated below Table 1 and Figures 2-6.

It was observed that known impurities are not co eluting with each other and main analyte peak.

Interference from degradation products

A study was conducted to reveal the effective separation of degradants/impurities from Levetiracetam. Sample solutions and placebo solutions were exposed to the following stress conditions to degradation. Stressed and unstressed samples were injected into the HPLC system with photo diode array detector by following test method conditions. All degrading peaks were resolved from Levetiracetam peak in the chromatograms of all samples and placebo did not show any interference at the retention time of Levetiracetam and impurities under the above conditions. The observations are tabulated below Table 2.

Levetiracetam was sensitive to stress condition like alkali. The results proved that the developed method has good selectivity and specificity, and is suitable for determination of impurities in Levetiracetam liquid dosage form.

Precision

System precision

System precision was demonstrated by prepared standard solution as per the test method and injected for six times into HPLC system. The retention time and area response of analyte peak were recorded. The observations are tabulated below Table 3.

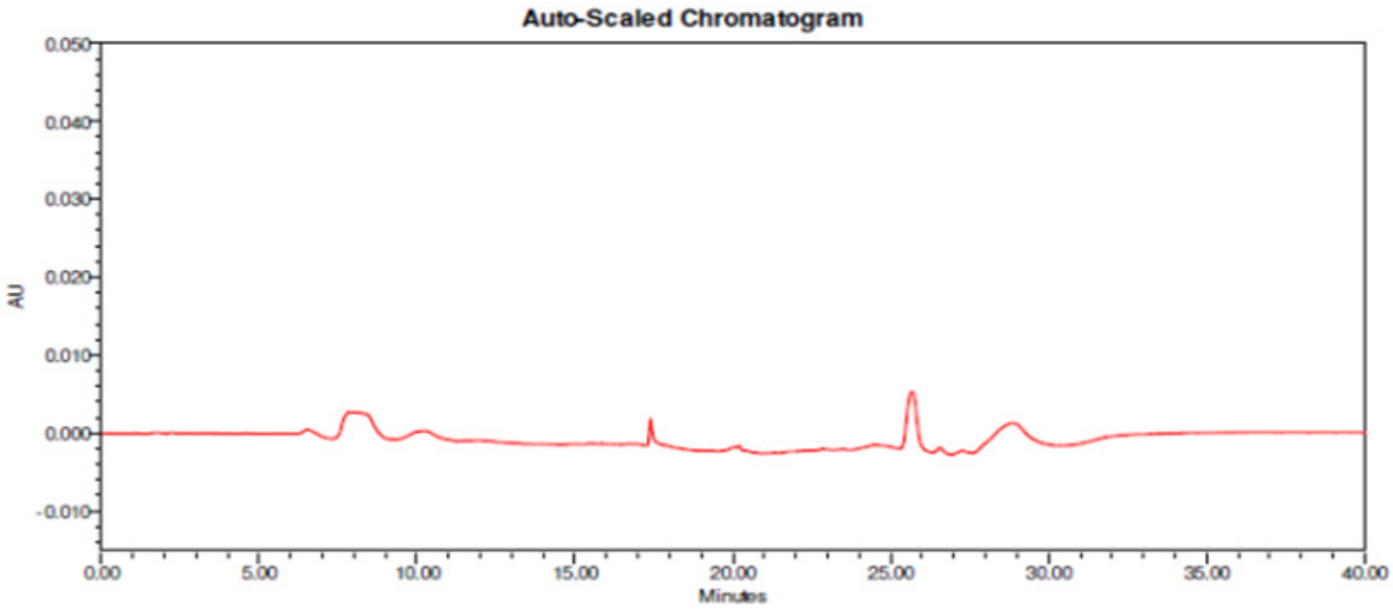

Figure 2: Typical chromatogram of blank.

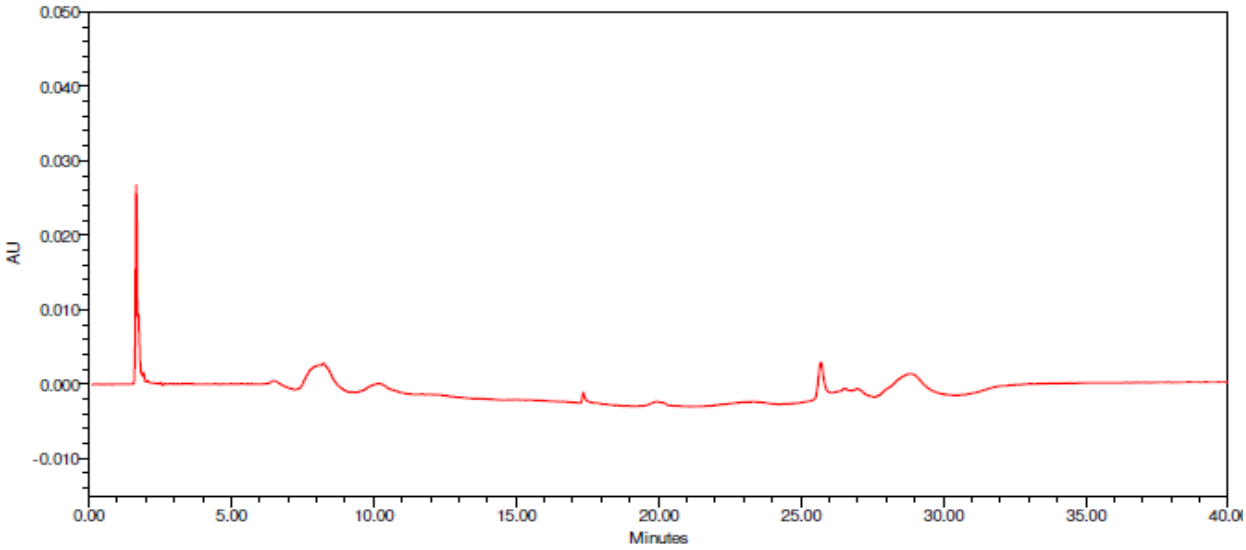

Figure 3: Typical chromatogram of placebo.

The %RSD of peak area for Levetiracetam standard was found to be 0.9% which is below 5.0% indicates that the system gives precise result.

Method precision

Method precision was demonstrated by prepared six control samples and six samples by spiking of impurities at specification level and analyzed as per the test method. The samples were prepared as per the method and the result for precision study is tabulated in Table 4 and Table 5.

Preparation of sample solution

Transferred 1 mL of sample solution into 20 mL of volumetric flask, containing 10mL of diluent, diluted to volume with diluent and mixed well.

Preparation of spiked sample solution

Transferred 1 mL of sample solution into 20 mL of volumetric flask containing 5 mL diluent and then added 1.0 mL of impurity-A stock solution, 0.17 mL of impurity-C stock solution, 0.34 mL of Levetiracetam RC-A stock solution made up to the volume with diluent and mixed well.

The results were well within the limits. From the above results, it is concluded that method is precise.

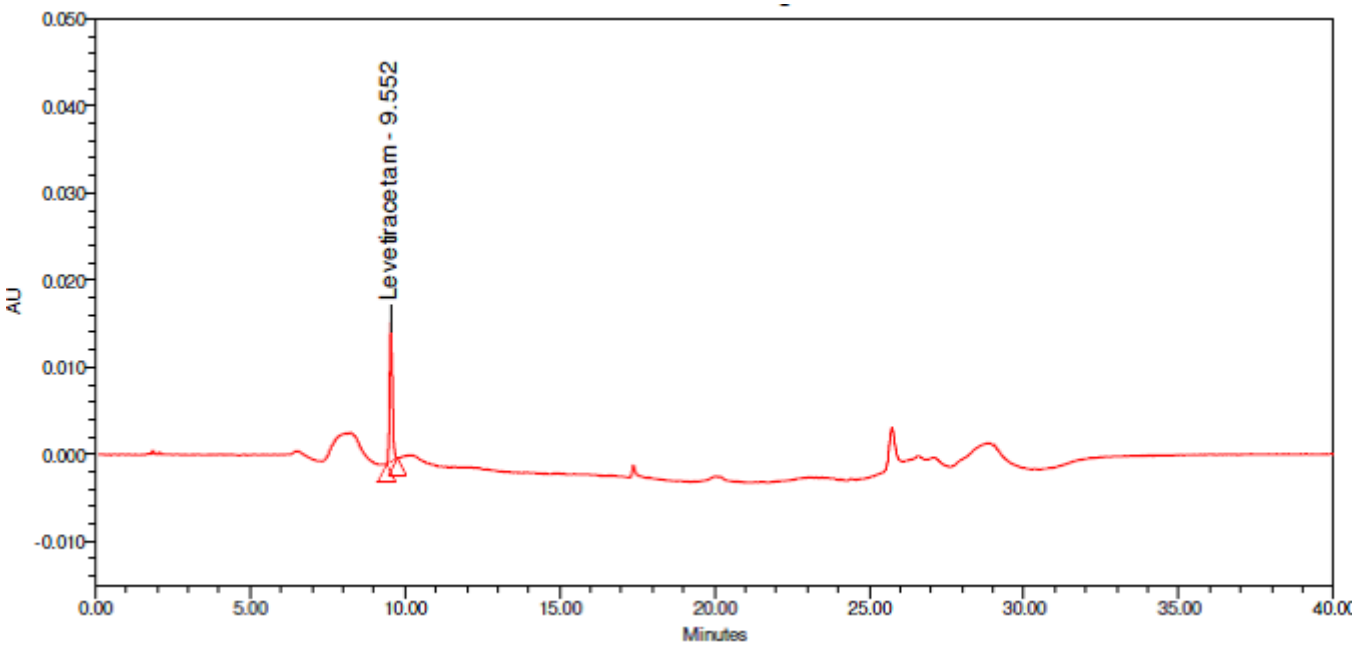

Figure 4: Typical chromatogram standard.

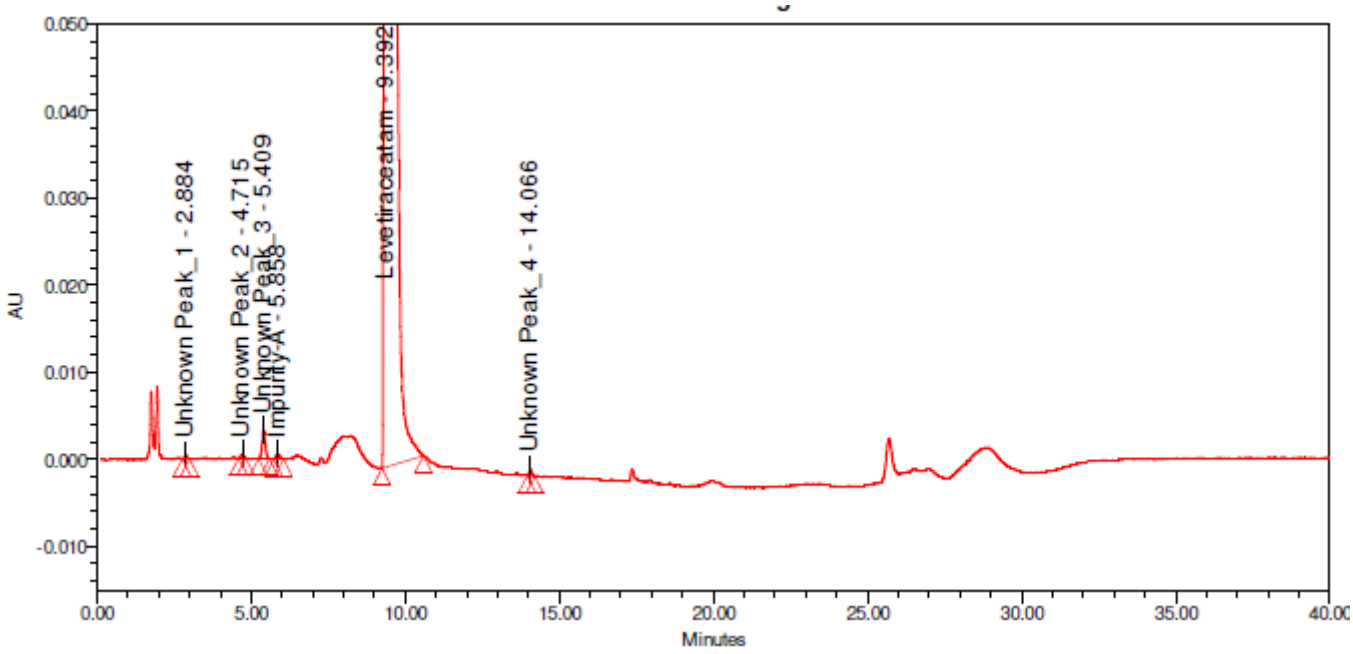

Figure 5: Typical chromatogram as such sample.

Limit of detection (LOD) and Limit of Quantitation (LOQ)

Limit of detection: The worst found signal to noise ratio for each peak was greater than 3 in each injection. All the peaks were detected in all the three injections.

Limit of Quantitation: The worst found signal to noise ratio for each peak was greater than 10 in each injection. All the peaks were detected in all the six injections. The observations are tabulated below Tables 6-7.

The limit of quantitation and limit of detection values obtained for each impurity and Levetiracetam are within the acceptance criteria.

Linearity

The linearity of detector response for analytes was demonstrated by preparing solutions over the range of 0.1%, 0.5% and 1.0% of specification limit with respect to sample concentration. These solutions were injected into the HPLC system and the responses of the same were recorded. A plot of concentration vs. peak area was done. The Coefficient of determination between

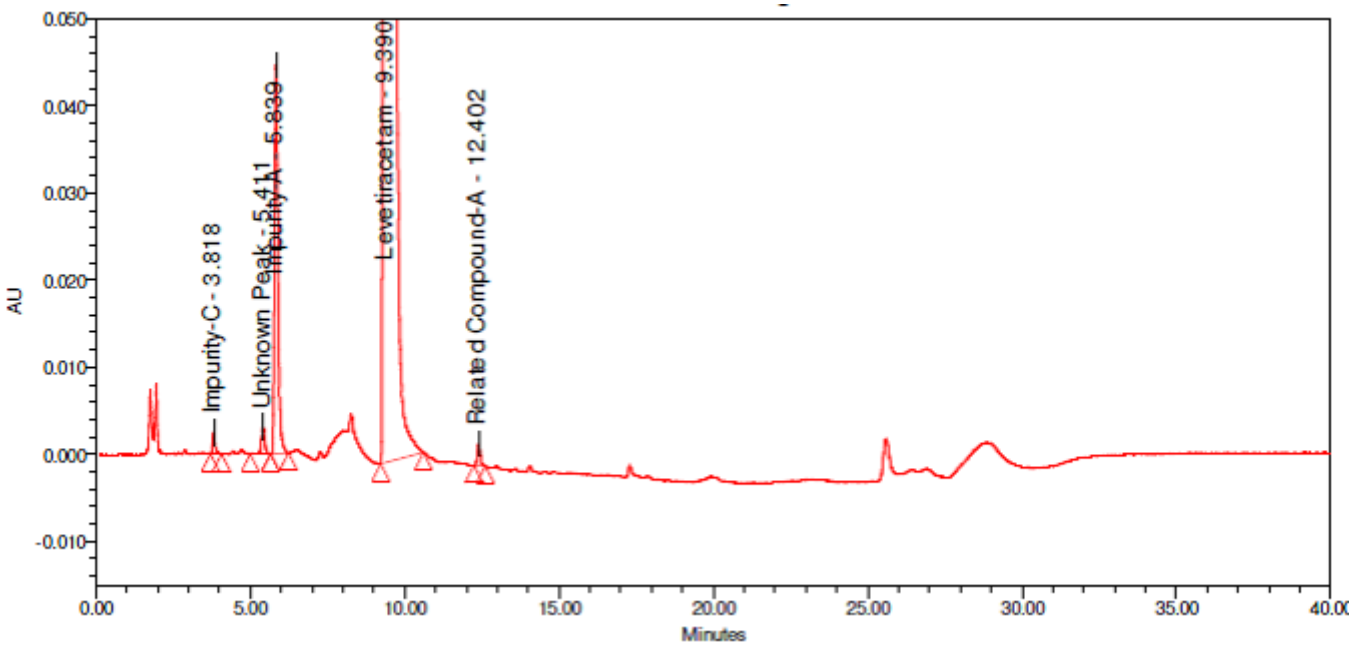

Figure 6: Typical chromatogram spiked sample.

Table 2: Forced Degradation results.

Sl. No.	Degradation	Assay (%)	Total Impurities (%)	Mass Balance (%)
1	Control sample	101.4	0.03	NA
2	Acid degradation (0.1N HCl/2mL/BT/17hr)	102.6	0.14	101.3
3	Base Degradation (1.0N NaOH/2mL/BT/1hr)	98.3	7.61	104.4
4	Peroxide Degradation (3% H_2O_2/2 mL/BT/17hr)	102.7	0.04	101.3
5	Thermal Degradation (60°C/ Thermal oven/44hr)	101.6	0.04	100.2
6	Water degradation (Water/2mL/BT/17hr)	99.7	0.03	98.3

Table 3: System precision data for Levetiracetam.

Sl. No.	Area response	Retention time
1	104942	9.590
2	103361	9.525
3	102261	9.552
4	104228	9.543
5	103407	9.545
6	103094	9.540
Average	103549	9.55
% RSD	0.90	0.23

concentration and response was evaluated. The observations are tabulated below Tables 8-11 and Figures 7-10.

The linearity results for Levetiracetam and all the impurities in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.

Accuracy

Recovery of Levetiracetam impurities in Levetiracetam was performed. The sample was taken and varying amounts of Levetiracetam impurities representing LOQ to 150 % of specification level were added to the flasks. The spiked samples were prepared as per the method and the results are tabulated in Table 12.

Table 4: Results of method precision (Control samples).

Sl. No.	Sample Details	Impurity-A (%)	Impurity-C (%)	RC-A (%)
1	Prep-1	0	ND	ND
2	Prep-2	0	ND	ND
3	Prep-3	0	ND	ND
4	Prep-4	0	ND	ND
5	Prep-5	0	ND	ND
6	Prep-6	0	ND	ND
Average		0	NA	NA
Std. Dev		0	NA	NA
% RSD		0	NA	NA

Table 5: Results of method precision (Spiked samples).

Sl. No.	Sample Details	Impurity-A (%)	Impurity-C (%)	RC-A (%)
1	Prep-1	105.5	103.7	94.2
2	Prep-2	105.1	96.3	103.8
3	Prep-3	105.1	103.7	96.2
4	Prep-4	104.8	100.0	96.2
5	Prep-5	104.8	103.7	92.3
6	Prep-6	105.1	92.6	94.2
Average		105.1	100.0	96.2
Std. Dev		0.2582	4.6802	4.0238
% RSD		0.20	4.7	4.2

Table 6: LOD and LOQ concentrations and S/N values for Levetiracetam and impurities.

Name of the impurity	Concentration in (%)		Signal to noise ratio value	
	LOD	LOQ	LOD	LOQ
Impurity-A	0.0049	0.0147	5	15
Impurity-C	0.0024	0.0074	4	10
Levetiracetam RC-A	0.0091	0.0277	6	20
Levetiracetam	0.0023	0.0070	4	16

Table 7: LOQ precision for Levetiracetam and impurities.

Sl. No.	Name of the solution	Impurity-A	Impurity-C	RC-A	Levetiracetam
1	LOQ precision-1	14144	3883	8416	6320
2	LOQ precision-2	13972	3554	8124	6617
3	LOQ precision-3	14653	3678	8256	6187
4	LOQ precision-4	13987	3450	7767	6410
5	LOQ precision-5	14261	3672	8139	5914
6	LOQ precision-6	14419	3802	8297	6188
Average		14239	3673	8167	6273
%RSD		1.9	4.3	2.7	3.8

Table 8: Linearity for Impurity-A.

Sl. No	Levels	Concentration in ppm	Area response
1	Linearity Level-1	4.98	112533
2	Linearity Level-2	24.91	561621
3	Linearity Level-3	49.82	1138634
Correlation coefficient (r^2)			1.000
Slope			22896.162
Intercept			-4101.311

Table 9: Linearity for Impurity-C.

Sl. No	Levels	Concentration in ppm	Area response
1	Linearity Level-1	5.36	57020
2	Linearity Level-2	26.81	288285
3	Linearity Level-3	52.84	575937
Square root of Correlation coefficient (r^2)			0.9999
Slope			10934.649
Intercept			-2782.636

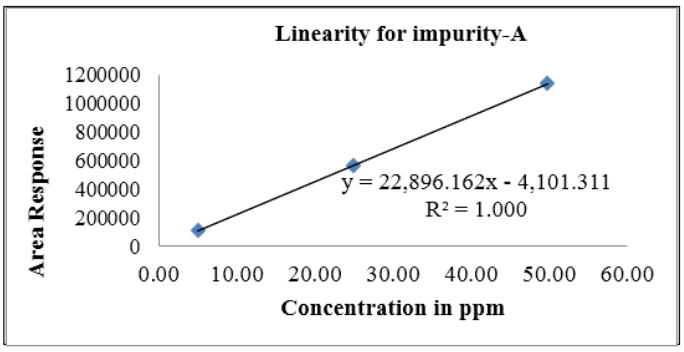

Figure 7: Linearity graph of Impurity-A.

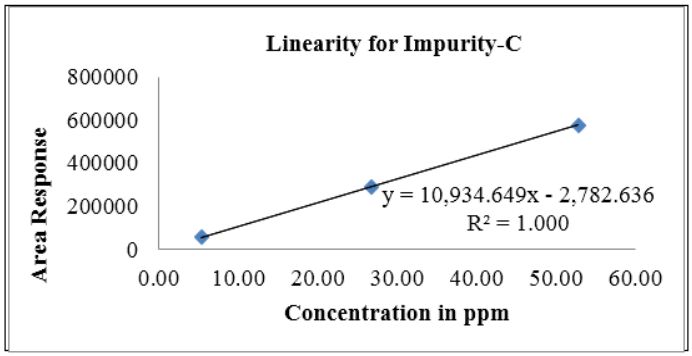

Figure 8: Linearity graph of Impurity-C.

Accuracy at LOQ level to 150% level for impurity-A, impurity-B and impurity-C is meeting the acceptance criteria. From the above results, it is concluded that method is accurate.

Solution stability of analytical solutions

Standard and sample and spiked sample solutions were kept for 48 hr at room temperature in transparent bottles in auto sampler and in refrigerator 2-8°C. The stability of standard and sample and spiked sample solutions was determined by comparison of "old" prepared standard solutions with freshly prepared standard solutions. The observations are tabulated below Tables 13-17.

From the above results, it is concluded that standard and sample solutions are stable up to 48 hr in both the conditions (bench top and refrigerator).

From the above results, it is concluded that spiked sample solutions are stable up to 48 hr in refrigerator condition.

DISCUSSION

A simple, economic, accurate and precise HPLC method was successfully developed. In this method it was carried out by using Inertsil ODS-3V, 150 x 4.6 mm, 3µm column and the mobile phase consists of two channels A and B. Channels A and B. channel-A: pH 5.50 phosphate buffer : acetonitrile (950:50 v/v) and channel-B: acetonitrile: water (90:10 v/v). The flow rate is 1.0 mL/min. The column temperature was maintained at 40°C and sample temperature was maintained at 25°C, injection volume 10µL and wavelength fixed at 205 nm. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of selectivity, accuracy, linearity, precision, and stability of solution.

For selectivity, the chromatograms were recorded for standard and sample solutions of Levetiracetam and its related substances. Selectivity studies reveal that the peak is well separated from each other. Therefore the method is selective for the determination of related substances in Levetiracetam. There is no interference

Table 10: Linearity for RC-A.

Sl. No.	Levels	Concentration in ppm	Area response
1	Linearity Level-1	5.22	33982
2	Linearity Level-2	26.11	178822
3	Linearity Level-3	51.46	358728
Correlation coefficient (r^2)			1.000
Slope			7025.956
Intercept			-3402.171

Table 11: Linearity for Levetiracetam.

Sl. No.	Levels	Concentration in ppm	Area response
1	Linearity Level-1	10.07	212864
2	Linearity Level-2	25.17	509508
3	Linearity Level-3	50.34	1051683
Square root of Correlation coefficient (r2)			0.9994
Slope			20901.460
Intercept			-4883.378

Table 12: Accuracy study of Levetiracetam.

Sl. No.	Theoretical (%)	% Mean Recovery		
		Impurity-A	Impurity-C	RC-A
1	LOQ	92.2	109.6	90.5
2	100	105.0	101.2	98.1
3	150	104.2	101.8	88.1

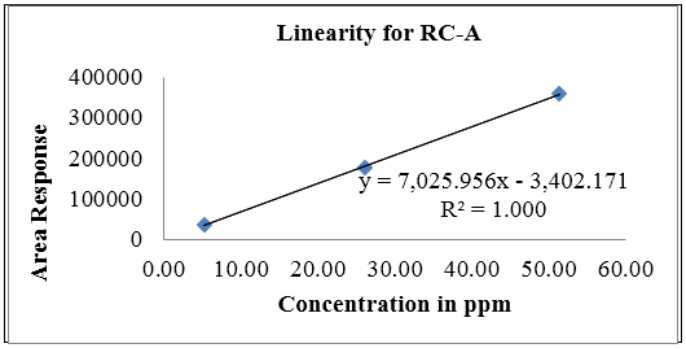

Figure 9: Linearity graph of RC-A.

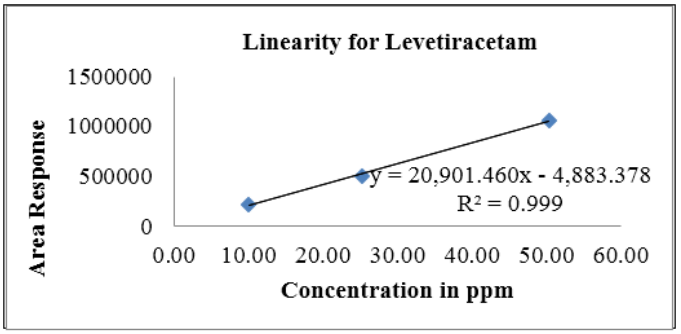

Figure 10: Linearity graph of Levetiracetam.

Table 13: Results for solution stability of standard.

Time Interval	%Recovery	
	Room temperature	Refrigerator
Initial	NA	NA
24 hr	99.1	101.4
48 hr	100.7	102.6

Table 14: Results for solution stability of test solution at room temperature.

Component	Initial	After 24 hr	% Difference	After 48 hr	% Difference
Impurity-A	ND	ND	NA	ND	NA
Impurity-C	ND	ND	NA	ND	NA
RC-A	ND	ND	NA	ND	NA
Maximum unknown impurity	ND	ND	NA	ND	NA
Total impurities	ND	ND	NA	ND	NA

Table 15: Results for solution stability of test solution at refrigerator.

Component	Initial	After 24 hr	% Difference	After 48 hr	% Difference
Impurity-A	ND	ND	NA	ND	NA
Impurity-C	ND	ND	NA	ND	NA
RC-A	ND	ND	NA	ND	NA
Maximum unknown impurity	ND	ND	NA	ND	NA
Total impurities	ND	ND	NA	ND	NA

Table 16: Results for solution stability of spiked sample at room temperature.

Component	Initial	After 24hr	% Difference	After 48 hr	% Difference
Impurity-A	Impurity-A	105.5	105.1	0.00	105.1
Impurity-C	Impurity-C	103.7	96.3	0.00	103.7
RC-A	RC-A	94.2	65.4	0.02	61.5

Table 17: Results for solution stability of spiked sample at refrigerator.

Component	Initial	After 24 hr	% Difference	After 48 hr	% Difference
Impurity-A	105.5	105.1	0.00	104.4	0.00
Impurity-C	103.7	103.7	0.00	96.3	0.00
RC-A	94.2	94.2	0.00	94.2	0.00

of diluent and placebo at Levetiracetam and impurities peaks. The elution order and the retention times of impurities and Levetiracetam obtained from individual standard preparations and mixed standard preparations are comparable.

The limit of detection (LOD) and limit of quantitation (LOQ) for Levetiracetam standard 0.0023% and 0.0070%, impurity-A 0.0049% and 0.0147%, impurity-C 0.0024% and 0.0074% and Levetiracetam RC-A 0.0091% and 0.0277% respectively.

The linearity results for Levetiracetam and all the impurities in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99. Calibration curve was plotted and correlation co-efficient for Levetiracetam and its impurities found to be 1.000, 0.9999, 1.000 and 0.9994 respectively.

The accuracy studies were shown as %recovery for Levetiracetam and its impurities at specification level. The limit of % recovered shown is in the range of 80 and 120% and the results obtained were found to be within the limits. Hence the method was found to be accurate.

For precision studies six replicate injections were performed. %RSD was determined from the peak areas of Levetiracetam and its impurities. The acceptance limit should be not more than 10, and the results were found to be within the acceptance limits.

CONCLUSION

The new HPLC method developed and validated for determination of Levetiracetam pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also

determining lower concentration of drug in its liquid dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

API: Active pharmaceutical ingredients; **HPLC:** High-Performance Liquid Chromatography; **HPTLC:** High-Performance Thin-Layer Liquid Chromatography; **UPLC:** Ultra-Performance Liquid Chromatography; **RT:** Retention Time; **ICH:** International council on Harmonization; **hr**: Hours; **SD:** Standard Deviation; **RSD:** Relative Standard Deviation.

REFERENCES

1. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry Part 2. 4th ed. CBS publishers and distributors. New Delhi, 1997;310-37.
2. Sethi P.D., HPLC: High Performance Liquid Chromatography Pharmaceutical Formulations, 1st edition, CBS publishers and distributors, New Delhi., 2001;3(39):116-20.
3. Patwekar SL, Sakhare RS, Nalbalwar NN. HPLC method development and validation-A general Concept. Int J Chem Pharm Sci. 2015;6(1):8-14.
4. Ravisankar P, Niharika A, Anusha H, Himaja V, Afzal Basha SK. A simple validated UV spectrophotometric method for quantitative analysis of levetiracetam in pharmaceutical dosage form. Indian J Res Pharm Biotechnol. 2015;3(5):380-5.
5. Lohita M, Shakirbasha S, Edukondalu T, Vijayaraj S, Jayapreethi P, Swetha K. Development and Validation of area under the curve and first derivative methods for the Estimation of levetiracetam in Bulk and Tablet Dosage Form by using UV spectrophotometry. J Pharm Biomed Anal Lett. 2014;2(2):134-40.
6. Muralikrishna Ch, Ramu G, Bikshambabu A, Venkata Rao S, Rambabu C. Spectrophotometric determination of levetiracetam by developing coloured complexes with 2-Chlorophenylhydrazine and anthranilic acid. Asian J Chem. 2012;24(4):1855-7.
7. Chaitanya Prasad MK, Sagar GV, Sudhakar P. Stability indicating RP HPLC method for determination of levetiracetam in pharmaceutical formulation. Indian J Pharm Biol Res. 2013;1(4):45-54. doi: 10.30750/ijpbr.1.4.9.
8. Usman M, Bibi H, Khalil MT, Hussain A, Siddiqua A, Iqbal J. Estimation of levetiracetam in bulk and pharmaceutical dosage form with a newly developed and validated RP-HPLC method. Res J Recent Sci. 2013;2(5):68-70.
9. Siddiqui FA, Sher N, Shafi N. Development of new method for simultaneous analysis of piracetam and levetiracetam in pharmaceuticals and biological fluids: application in stability studies. BioMed Res Int. 2014:1-8.
10. Nagaraju P, Priyadarshini GI, SCHVSS. Appaji. Development and validation of novel HPLC method for estimation of levetiracetam in pharmaceutical formulations. Int J Pharm Chem Sci. 2014;3(2):522-7.
11. Lakshmana Rao A, Naga Jahnavi V. A validated RP-HPLC method for the estimation of levetiracetam in bulk and pharmaceutical formulations. E-journal of chemistry. 2010;7(2):600-4.
12. Tyagi MG, Kannan S, Manickam AS, Chander J. A novel HPLC estimation technique for the antiepileptic Drug levetiracetam in tablet and human urine samples. World J Pharm Pharm Sci. 2015;4(6):1093-9.
13. Devanaboyina N, Satyanarayana T, Rao BG. A novel RP-HPLC method for the analysis of levetiracetam in formulations. Pharm Chem. 2011;3(6):112-7.
14. Gopalakrishnan S, Jeyashree B, Ramalakshmi A, Chenthilnathan A. Analytical method development and validation for the determination of levetiracetam in pharmaceutical formulations by using RP-HPLC. Pharm Lett. 2011;3(3):194-201.
15. Valarmathy J, Samueljoshua L, Rathinavel G, Selvin Thanija C. T Sivakumar. RPHPLC method development and validation for assay of levetiracetam in tablet dosage form. Res J Pharm Technol. 2008;1(3):395-7.
16. Rao BM, Ravi R, Shyam Sundar Reddy BS, Sivakumar S, Gopi Chand I, Praveen Kumar K, *et al*. 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. J Pharm Biomed Anal. 2004;35(5):1017-26. doi: 10.1016/j.jpba.2004.03.015, PMID 15336349.
17. Can NO, Arli G. Reversed-phase HPLC analysis of levetiracetam in tablets using monolithic and conventional C_{18} silica columns. J AOAC Int. 2010;93(4):1077-85. doi: 10.1093/jaoac/93.4.1077, PMID 20922937.
18. Hadad GM, Abdel Salam RA, Abdel Hameed EA. Quantitative determination of levetiracetam in human urine using HPLC-UV and its identification by LC-ESI-MS. J Liq Chromatogr Relat Technol. 2013;36(18):2568-79. doi: 10.1080/10826076.2012.723091.
19. Karas K, Kuczynska J, Sienkiewicz-Jarosz H, Bienkowski P, Mierzejewski P. A simple bioanalytical method for the quantification of levetiracetam in human plasma and saliva. J Chromatogr Sep Tech. 2015;6(7):1.
20. Ramprasad MVNS, Syama Sundar B. Development and validation of high performance liquid chromatographic method for the determination of levetiracetam in human plasma by Electospray ionization-mass spectrometry. Int J Sci Eng Res. 2014;5(3):602-9.
21. Shihabi ZK, Oles K, Hinsdale M. Analysis of the antiepileptic drug Keppra by capillary electrophoresis. J Chromatogr A. 2003;1004(1-2):9-12. doi: 10.1016/s0021-9673(03)00716-7, PMID 12929956.

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