https://doi.org/10.48047/AFJBS.6.13.2024.5634-5644

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Robust Stability Indicating Analytical Method for Levetiracetam in Tablet Dosage Form

Vijaya Lakshmi Marella[1*], Suneetha Achanti[1], InduriMadhu Sudhan Reddy [2], Kanaka Durga Devi Nelluri[1], Kiranmai seelam[1], Chetan Mokkapati[1], Kolaventi BhuvanaLekha[1]

[1]Department of Pharmaceutical Analysis, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, Andhra Pradesh, India

[2]Department of Pharmaceutical Chemistry&Analysis,SVS College of Pharmaceutical Education and Research,Tembhurni, Tal-Madha, Dist- Solapur: 413211,Maharashtra, India

Correspondence :Dr. Vijaya Lakshmi Marella

Associate Professor**,** Department of Pharmaceutical Analysis, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada - 520 010, Andhra Pradesh, India

E-mail: analysis.scops@gmail.com

Volume 6, Issue 13, Aug 2024

Received: 15 June 2024

Accepted: 25 July 2024

Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.13.2024.5634-5644

ABSTRACT

A novel and highly selective reverse phase liquid chromatography (HPLC) method was optimised for quantifying levetiracetam in pharmaceutical formulations. The chromatographic resolution was achieved using a Symmetry C18 column (4.6 mm × 250 mm, 5 µm particle size) and a mobile phase consists of 0.1% formic acid in 70% aqueous solution and 30% methanol, flowing for 1.0 mL/min. Detection was performed at 210 nm. The full run time for the analysis was six minutes, and the retention time observed for levetiracetam was 4.405 minutes. Peak purity analysis conducted under all degradation conditions confirmed the homogeneity of the peak, thereby establishing the method as stability-indicating. The results of validation parameters demonstrated that the procedure exhibits linearity, precision, accuracy, reproducibility, and selectivity for the analysis of levetiracetam.All obtained results met acceptance criteria, confirming the acceptability of the approach for routine analytical control and drug assay purposes.

Keywords: Levetiracetam, RP-HPLC, Stability indicating method, ICH Guidelines

Running title: Novel Analytical method for levetiracetam

INTRODUCTION

In this study, the focus is on Levetiracetam (LEV) analysis, a novel antiepileptic drug used for treating tonic-clonic and myoclonic seizures. The chemical name for LEV is (-)-(S)-α-ethyl-2-oxo-1-pyrrolidine acetamide, and its formula is $C_8H_{14}N_2O_2$. It is highly soluble in water and chloroform, soluble freely in methanol and ethanol, soluble sparingly in

acetonitrile, and is insoluble practically in n-hexane. One of the advantages of LEV is its favourable pharmacokinetic properties. These characteristics comprise 100% oral bioavailability, linear pharmacokinetics, minimal protein binding, absence of hepatic metabolism, rapid attainment of steady-state concentrations, low likelihood of drug interactions, and a half-life ranging from 6 to 8 hours [1-6]. Several chromatographic methods[17], such as HPLC[7-14,18] and LC-MS/MS[15-16], developed for the quantification of LEV in bulk and pharmaceutical dosage forms. The study aims to approach a HPLC method that is fast, sturdy, responsive, specific and reproducible for the quantification of LEV. The developed conditions were validated according to ICH guidelines[19], and the linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ) were determined. The method was used to quantify the drug content of LEV in various commercial dosage forms.

MATERIALS AND METHODS

Materials: For this study, the working standard levetiracetam (LEV) was obtained from Dr. Reddy's Laboratories Ltd. in Hyderabad. HPLC grade methanol, acetonitrile and water were procured from Merck Life Science Private Limited in Mumbai, India. All other chemicals and solvents of analytical grade and procured from Loba Chemie in Mumbai, India.

Instrumentation: The analytical system used in this study was the Shimadzu LC-10AT VP system, which includes a binary gradient pump, a UV-Vis SPD-10A VP detector, an inbuilt SIL-20A HT autosampler with loop volume, with a column and data acquisition with LC Solution software. To enhance the dissolution of the analyte in the diluent, a Cyclo Mixer (Remi Model: CM 101) was used. The mobile phases were degassed using an Ultra Sonicator (Loba Life; Model: 3.5L 100). pH adjustment was done using a digital pH meter (Systronics µ pH System 361), and all materials were weighed using a digital balance (Amkette Shimadzu; Model: ATX224).

Preparation of Solutions:

Standard Stock solution preparation:*T*o prepare the solution, 100mg of LEV was exactly weighed and taken into a 100mL volumetric flask. HPLC grade water was added to the flask, and the solution was shaken vigorously. The volume was then finalised to the mark with the HPLC water to get 1000 µg/mL. It was subsequently passed though 0.45µm membrane filter before use.

Sample preparation for the assay of levetiracetam in the tablets: To create the sample solution, twenty tablets underwent weighing and were subsequently pulverized into a fine powder using a mortar.A precisely measured amount of powder, equal to 100mg, was subsequently added to a 100mL calibrated flask already containing 50mL of high-performance liquid chromatography (HPLC) water. The solution underwent sonication to facilitate the dissolution of the powder, followed by topping up the remaining volume with water to reach the flask's mark. Subsequently, the obtained solution was passed through a 0.45µm nylon membrane filter. Following that, 15µL of the previously prepared solution were carefully pipetted into a 1mL vial, and the remaining volume was made with diluent to achieve 15µg/mL. This final solution was then introduced into the high-performance liquid chromatography (HPLC) in triplicate, employing optimized chromatographic conditions.

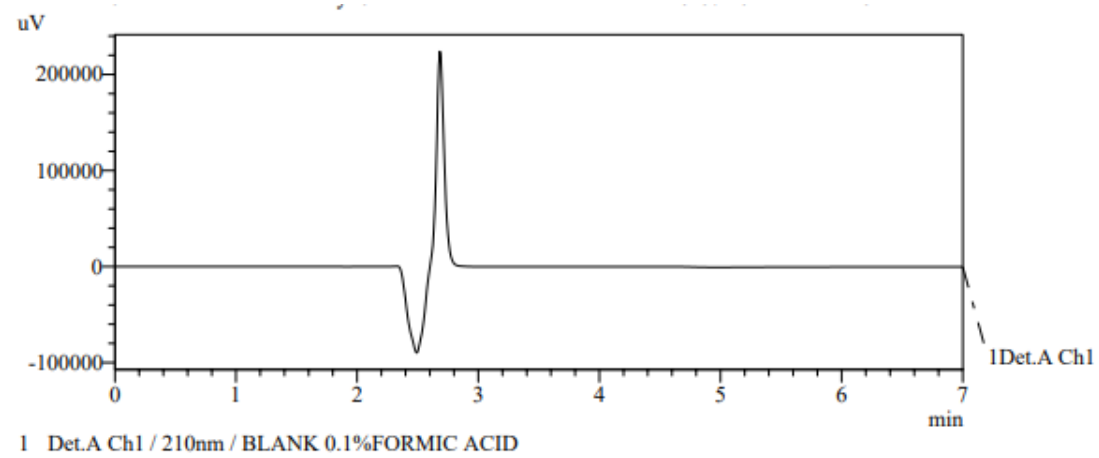
Validation of the method: The developed methods was validated according to International Conference on Harmonisation (ICH) guidelines Q2 (R1).[17]

Forced degradation studies: During forced degradation studies, standard LEV samples were exposed to thermal, basic, peroxide, acidic and photolytic conditions. For acidic studies of degradation, the stock solution was mixed with 0.1 N hydrochloric acid (HCl) and heated under reflux at 60°C for half an hour. The mixture was then balanced for neutralisation with the addition of 0.1 N sodium hydroxide (NaOH). For basic degradation, the stock solution was mixed with 0.1 N sodium hydroxide and heated under reflux at 60°C for 30 minutes. The mixture was balanced by adding 0.1 N HCl. To induce oxidative break down conditions, the stock solution was combined with 3% v/v hydrogen peroxide (H2O2) and maintained at 60°C for 30 minutes. To degrade thermally, the standard LEV was subjected to exposure at 105°C for 1 hour. For degradation of the drug photolytically, the powdered drug was placed to UV light for 1 hour. For every study of degradation, corresponding solutions without drug were made following the same procedure described above, omitting the addition of standard LEV. After completing the degradation study, the solutions returned to room temperature and then made with diluent to achieve 15 µg/mL. Before injecting, all solutions were passed through using 0.45 µm membrane filter.

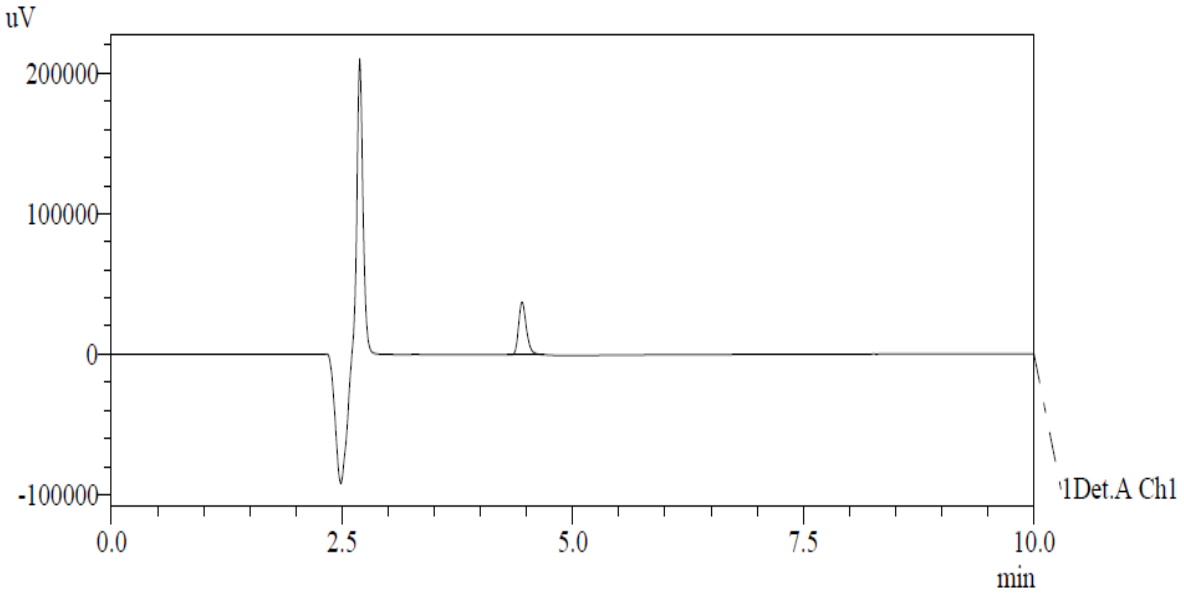
RESULTS AND DISCUSSION

Optimal method design: The following steps are essential for developing a chromatographic method. Specifically, attention must be given to standardize sample preparations, selecting the mobile phase, choosing the stationary phase, and deciding on the detector. With the help of Phenomenex column C18 (250 length x 4.6 Dia mm, 5µm particle size) and a mobile

phase consists of 0.1% formic acid and methanol (70:30% v/v) at the rate of 1.0 mL/min, an acceptable resolution with well defined peaks and a stable base line was accomplished. Levetiracetam (LEV) quantitation was performed at 210 nm. A typical chromatogram (Fig. 1) generated by the approached RP-HPLC method illustrates the symmetrical peak of LEV. The observed time of retention (4.405 min) allows for rapid drug determination, making it suitable for routine quality control analysis.



A) Blank



B) Standard LEV

Figure 1: An Optimized Chromatogram of A) Blank and B) Standard LEV

Table 1: Optimal analytical conditions

Parameters	Refined conditions
Column	C_{18}Column (250 x 4.6 mm; 5 µm)
Rate of flow	1.0 mL/min
Temp. of the oven with column	30^{o}C
Run Time	10 min.
Volume of injection	20 µL

Wavelength	210 nm
Mobile Phase	0.1% Formic acid: methanol (70:30% v/v)

Method Validation:

The system suitability assessment specifies that the peak asymmetry of the analyte should not exceed 1.5, the theoretical plates number for the sample peak must be more than 2000, and the deviation in the standard relatively for 5 duplicate injections of the standard preparation should be less than 2.0%. The data given in Table 2 demonstrate that the instrument was deemed appropriate to the intended analysis.

Table 2: Results of system suitability parameters

Parameter*	LEV	Limit
Peak Area (%RSD)	0.841	NMT: 2 %
Retention Time (%RSD)	0.542	NMT: 1 %
Tailing Factor	1.341	NMT: 1.5
No. of Theoretical Plates	11669	NLT: 2000

** Replicates of five determinations; RSD: Relative Standard Deviation; NMT: Not More Then; NLT: Not Less Then*

The linearity results presented in this study illustrate the satisfactory linearity of drug (LEV) across 50% to 150% range of the target concentration (refer to Fig. 2). A linearity was established for the area under the curve and the quantity of LEV within 5 to 25 µg/mL range. The correlation was (r^2: 0.9991) obtained and the regression line indicates good association for the area under the peak and the quantity of LEV. Details for the regression line is provided in Table 3.

Table 3: Data to show linearity

Conc. (µg/mL)	Peak Area (Mean ± SD)*	RSD (%)	Linear regression equation
5	128422 ± 1173	0.913	y = 26863x +1296.3 R^2 = 0.9991
10	275036 ± 983	0.357	
15	409014 ± 1835	0.449	
20	542449 ± 6079	1.121	
25	666293 ± 7542	1.132	

**Replicates of three injections; SD: Standard Deviation; RSD: Relative Standard Deviation*

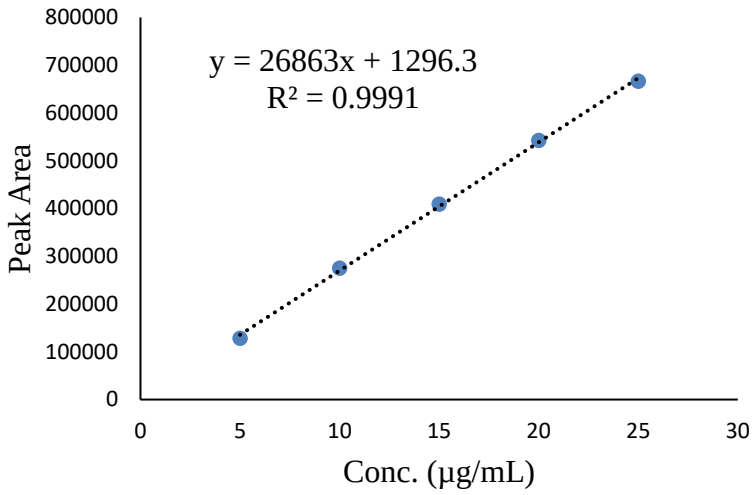


Figure 2:Proposed method:Calibration curve for LEV.

The detection limit (LOD) and quantification limit (LOQ) were quantified based on the standard deviations of the intercepts and slopes of the linear regression curves. For LEV, the LOD determined to be 0.34 µg/mL, and the LOQ found to be 1.04 µg/mL. From the recovery data has been confirmed that the percentage recovery was within the range (*i.e*. 98 to 102 %) and additionally%RSD values were also below 2.0%. Hence, the data proves that the developed approach is recovered good (Table 4).

Table 4: Study of recovery by standard addition method

Ana-lyte	Amount of standard drug spiked % Spiked	Quantity (mg)	Amount of sample taken (mg)	Recovery (Mean ± SD)	RSD (%)	%Recovery
LEV	50	50	100	49.82± 0.397	0.797	99.63
	100	100	100	100.21± 0.326	0.325	100.21
	150	150	100	150.58 ± 0.555	0.369	100.39

**Replicates of three determinations; SD: Standard Deviation; RSD: Relative Standard deviation*

The reproducibility of the method, assessed for precision, was estimated by calculating the relative standard deviation (%RSD) from six estimations of a 15 µg/mL of LEV conducted during the day and under same experimental conditionsThe relative standard deviation (RSD) value obtained from the reproducibility study was found to be less than 2%, indicating that the optimised method demonstrates good repeatability.Intermediate precision was evaluated by analyzing a single concentration with triplicates during the day (intra-day) and on three alternate days (inter-day). The percent RSD values obtained were 1.215% for intra-day precision and 1.439% for inter-day precision. This assessment demonstrates consistent and reliable performance of the method under varying experimental conditions.The relative

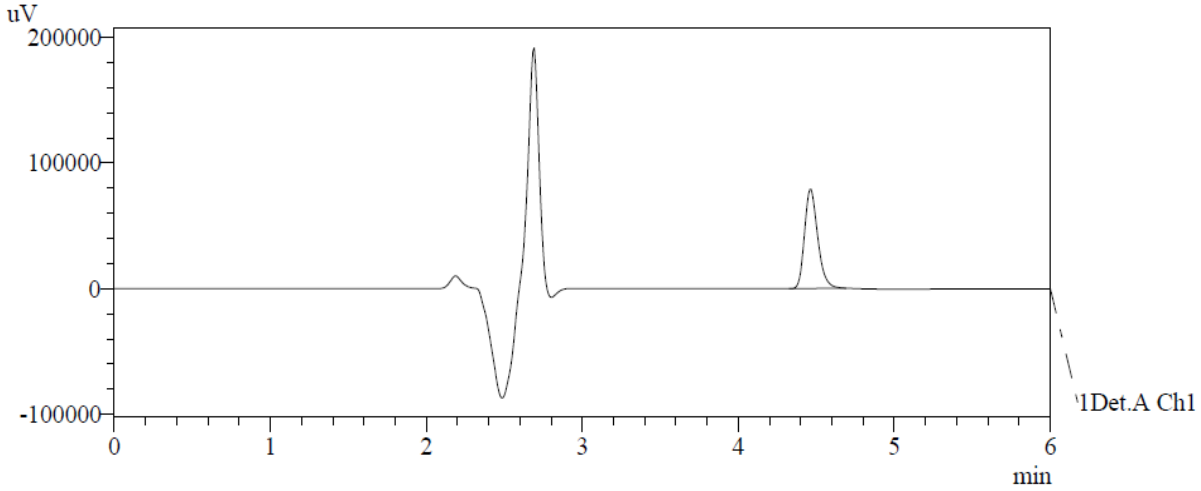
standard deviation (RSD) values for intermediate precision were determined to be less than 2%, indicating that the proposed methods exhibit reproducibility.The outcomes and the range of experimental variations assessed in the robustness study are detailed in Table 5. No notable alterations in the chromatograms were observed when small variations were applied to the optimised conditions, confirms the approach robustness.

Table 5: Results for Robustness Study

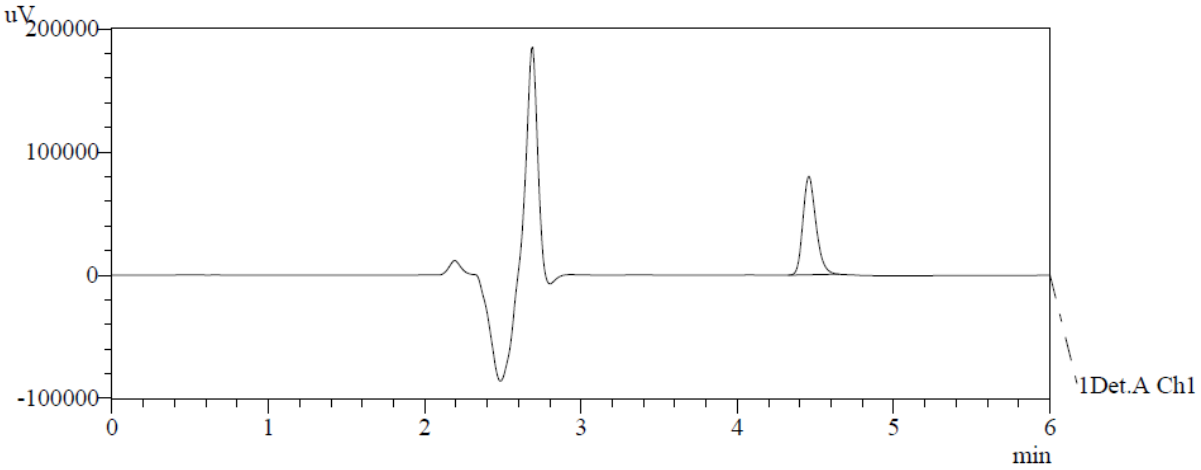
Parameter	Used	Retention Time*	Tailing Factor*	No. of Theoretical Plates*
Flow Rate (varied by ± 0.1 mL)	0.9	4.926	1.327	12177
	1.0	4.405	1.341	11664
	1.1	4.063	1.352	10822
Mobile Phase (varied by ± 5%)	65:35	3.847	1.383	10063
	70:30	4.406	1.351	11375
	75:25	5.407	1.288	13087

**Replicates of three determinations*

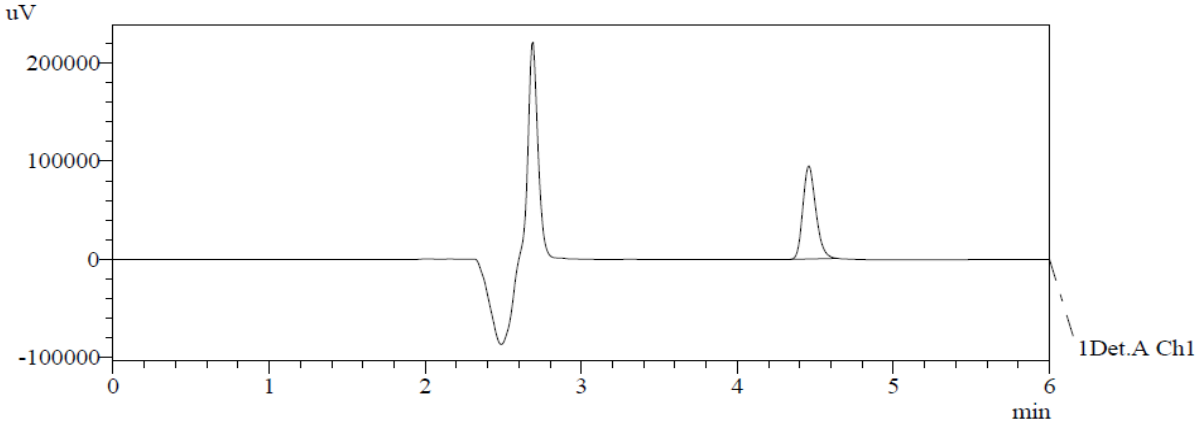
Compelled studies for degradation were conducted for assessing the stability-confirming properties of the analytical method, especially in cases where information regarding potential degradation products was lacking. The acceleratig test studies demonstrated that the procedure exhibited high specificity in detecting levetiracetam, effectively distinguishing it from potential degradation products.The drug exhibited degradation under photolytic conditions.Photolysis resulted in a peak with increased peak area at 7.4 mins (Figure 3). The forced degradation studies under acid, alkali, thermal, and oxidation conditions for levetiracetam (LEV) showed only a minor decrease in peak area with no detectable degradation products. The data of the stability for LEV under each stress condition are summarized in Table 6. The purity index values are consistently more than 0.9999, indicating high method selectivity



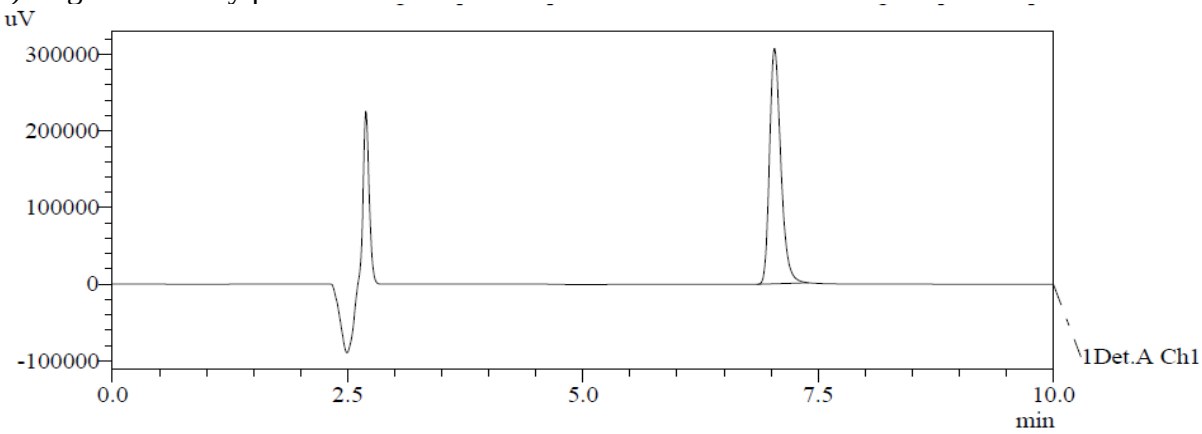
a) Degradation by acid



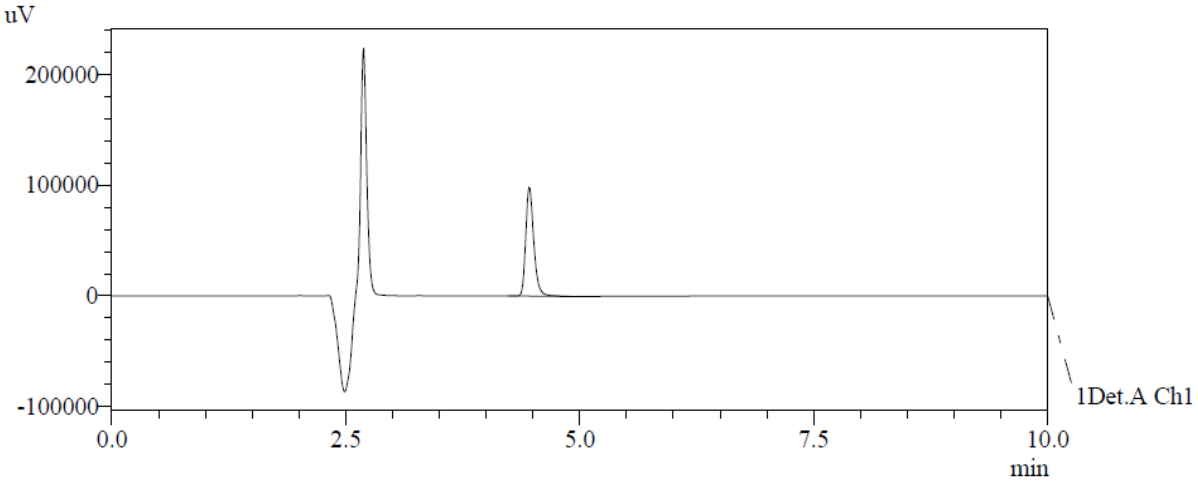
b) Degradation by alkali



c) Degradation by peroxide



d) Photolytical Degradation



e) Degradation thermally

Figure 3: Chromatograms of forced degradation samples of Levetiracetam (a) Acidic (b) Basic (c) Oxidation (d) Photolytic (e) Thermal

Table 6: Degradation study of Levetiracetam at different stress conditions.

Conditions	**Retention time (mins)**	**Area**	**Tailing factor**	**%Degradation**
Acidic Condition	4.458	463437	1.323	13.10
Basic condition	4.453	462269	1.312	12.81
Hydrogen Peroxide (3%)	4.453	446391	1.297	9.14
Photolytic Condition	7.033	566422	1.357	38.48
Thermal Condition	4.458	487854	1.353	19.27

Assay of levetiracetam in tablets: The developed reverse phase HPLC method can be utilized to quantifying levetiracetam (LEV) tablet formulation. The analysis presented in Table 7 shows the excellence of the assayed analytes and validate the suitability of method to routine evaluation of levetiracetam in tablet dosages.

Table 7:Results for table dosage form analysis

Brand Name	Label claim (mg)	% Analyte Estimated (Mean ±SD)*	RSD (%)
Levipil 250	250	101.05±0.332	0.329
Levipil 500	500	100.45±0.988	0.984

**Replicates of three determinations; SD: Standard Deviation; RSD: Relative Standard Deviation*

CONCLUSION

A reproducible and stability-assuring RP-HPLC technique has been established and verified for assessing LEV in tablet formulation. Validation findings affirmed the precision, accuracy, specificity, and robustness of the RP-HPLC approach. Successfully employed, this method is recommended for the quantitative evaluation of LEV in the formulations, especially in Quality Control settings where efficiency and time management are crucial.

ACKNOWLEDGEMENT

The authors express their gratitude to Siddhartha Academy of General and Technical Education for providing the necessary resources for this project

REFERENCES

1. Indian Pharmacopoeia, Ministry of Health and Family Welfare, Government of India, The Indian Pharmacopoeial Commission, Ghaziabad. 2018; Vol. 2: 2407-12.
2. Lyseng-Williamson KA. Spotlight on levetiracetam in epilepsy. CNS Drugs. 2011; 25(10): 901-5.
3. Sarah J Nevitt, Maria Sudell, Sofia Cividini, Anthony G Marson, Catrin Tudur Smith. Antiepileptic drug monotherapy for epilepsy: a network meta-analysis of individual participant data. Cochrane Database of Systematic Reviews. 2017; 6(6): CD011412.doi: 10.1002/14651858.CD011412.pub4.
4. Noachtar S, Andermann E, Meyvisch P, Andermann F, Gough WB, Schiemann-Delgado J; N166 Levetiracetam Study Group. Levetiracetam for the treatment of idiopathic generalized epilepsy with myoclonic seizures. Neurology. 2008; 70(8): 607-16.doi: 10.1212/01.wnl.0000297512.18364.40.
5. S. Poongothai, R. Srinivasan, M. Rama. A Correlative Dissolution Study of Oral Drug Delivery Systems through Reverse Phase-HPLC Analysis. International Journal of Pharmaceutical Quality Assurance. 2023; 14(3): 553-558.
6. Arshed A. Khalid, Wedad K. Ali. Controlled Release Levetiracetam Loaded Eudragits Microspheres for Oral Drug Delivery: Preparation and Evaluation. International Journal of Drug Delivery Technology. 2023; 13(1): 167-172.doi: 10.25258/ijddt.13.1.26.
7. Birbal Singh , Rita Santhakumar , Indu Bala , Shyam Baboo Prasad , Surajpal Verma Development and Validation of RP-HPLC Method for the Dissolution and Assay of Etoricoxib in Pharmaceutical Dosage Forms International Journal of Pharmaceutical Quality Assurance 2014; 6(1); 1-7
8. N. Appala Raju, J. Venkateswara Rao, K. Vanitha Prakash, K. Mukkanti, and K. Srinivasu.Estimation of levetiracetam in tablet dosage form by RP- HPLC. Journal of Chemistry. 2008; 5: Article ID 965430. Doi:10.1155/2008/965430.
9. Lakshmana Rao and V. Naga Jahnavi, A Validated RP- HPLC method for the estimation of levetiracetam in bulk and pharmaceutical formulations. e-J Chem. 2010; 7(2); 600-4.
10. Büşra Kandilli, Afife Büşra Uğur, Meltem Çetin, and Fatma Demirkaya Miloğlu. A Simple HPLC- UV Method for Simultaneous Determination of Levetiracetam and Carbamazepine.Hacettepe University Journal of Faculty Pharmacy. 2018; 38(2): 58-64.
11. Nafiz O Can, Goksel Arli. Reversed-Phase HPLC analysis of levetiracetam in tablets using monolithic and conventional C_{18} silica columns. J AOAC International. 2010; 93(4): 1077–85.doi:10.1093/JAOAC/93.4.1077.
12. Manuela Contin, Susan Mohamed, Fiorenzo Albani, Roberto Riva, Agostino Baruzzi, Simple and validated HPLC- UV analysis of levetiracetam in deproteinized plasma of

patients with epilepsy. Journal of Chromatography B.2008; 873(1): 129-32.doi: 10.1016/j.jchromb.2008.08.007.

13. Kiran Gawale and Shoaeb Mohammad Syed. Development and validation of UV spectroscopic and RP- HPLC method for determination of levetiracetam in bulk and combined dosage form**.** Journal of Pharmaceutical and Applied Chemistry. 2021; 7(1): 25-32.
14. Muhammad Usman, Hafsa Bibi, Muhammad Tariq Khalil, Altaf Hussain, Aisha Siddiqua and Javeid Iqbal. Estimation of levetiracetam in bulk and pharmaceutical dosage form with a newly developed and validated RP- HPLC method. Research Journal of Recent Sciences. 2013; 2(5): 68-70.
15. NuttawutJenjirattithigarn,NatthaWorachat, SuchawadeeHorsuwan, ApichayaPuangpetch, ChatchayPrempunpong,ChaiyosKhongkhatithum,LunliyaThampratankul, ntiratPrommas, AnannitVisudtibhan, ChonlaphatSukasem. Determination of plasma Levetiracetam level by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS-MS) and its application in pharmacokinetics studies in neonates. Journal of Chromatography B. 2018; 1085: 13-20.doi: 10.1016/j.jchromb.2018.03.037
16. Shan Zhou, Ruitong Li, Ziyi Chen, Ruina Ren, Xueding Wang, Qilin Dai, Dingshen Wen, Yanping Guan, Xiaoxu Zhang, Siyuan Tang, Liemin Zhou, Min Huang. LC–MS/MS quantification of levetiracetam, lamotrigine and 10-hydroxycarbazepine in TDM of epileptic patients. Biomedical Chromatography. 2022; 36(8); e5393. doi: 10.1002/bmc.5393.
17. Gaikwad, A. V. (2022). A High Performance Liquid Chromatography Method Development and Validation for the Estimation of Canagliflozin in Bulk and Marketed Dosage Form. Asian Journal of Pharmaceutics (AJP), *15*(04). https://doi.org/10.22377/ajp.v15i04.4230
18. Valarmathy J, Samueljoshua L, Rathinavel G, Thanuja C Selvin, Sivakumar T. RP-HPLC method development and validation for assay of levetiracetam in tablet dosage form. Research Journal of Pharmacy and Technology. 2008; 1(3): 395-7.doi: 10.5958/0974-360X.
19. International Conference on Hormonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedure: Text and Methodology {ICH- Q2 (R1)}; November 2015: 1-13.