IJBPAS, May, 2022, 11(5): 2571-2581

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

RP-HPLC ESTIMATION OF LEVETIRACETAM IN BULK AND FROM ITS FORMULATION

BHOSALE AP*, GHOTANE RB AND PATIL SV

Department of Quality Assurance, Ashokrao Mane College of Pharmacy, Pet-Vadgaon, Maharashtra 416112

***Corresponding Author: Aishwarya P. Bhosale: E Mail: aishubhosale1010@gmail.com**

Received 19th May 2021; Revised 18th July 2021; Accepted 10th Aug. 2021; Available online 1st May 2022

https://doi.org/10.31032/IJBPAS/2022/11.5.6117

ABSTRACT

The intention of current explore was to develop and validate suitable RP-HPLC method for analyzing Levetiracetam in single and combined dosage form as per ICH Guidelines. Separation was completed by using mobile phase consisting of HPLC grade water and acetonitrile in a proportion of 50:50. The separations were carried out on a Agilent Zorbax SB-Aq (250 x 4.6 mm, 5µ) at a flow rate of 1 mL/min. The injection volume was 10 µl and the peaks were detected at 205 nm. The calibration graph was plotted with concentration of the drug against the peak area was found to be linear in the range of 20-30µg/ml and coefficient of correlation was found to be 0.9989. In the accuracy study of developed method the percentage recovery of levetiracetam ranging from 99.81-100.47.The %RSD value was less than 2.0 for intraday and interday precision indicated that the method was highly precise. Linearity range was observed in concentration range 20-30 µg/ml. The Limit of Detection and Limit of Quantitation was found to be 2.20µg/ml and 6.66µg/ml respectively. The system suitability parameters for the developed method found as, the average asymmetry factor found to be 0.992 which indicates asymmetric nature of peak. The average number of Theoretical plates was found to be 9154 which indicates efficient performance of the column. Hence, the developed RP-HPLC method was reliable, linear, accurate, specific method.

Keywords: Levetiracetam, Accuracy, Precision, Estimation, Validation, Tablets, RP-HPLC

INTRODUCTION

Levetiracetam is a broad-spectrum antiepileptic drug approved by US food and drug administration in 1999. It is ethyl derivative of the nootropic drug piracetam **[1]**. Chemical name of Levetiracetam is (2*S*)-2-(2-oxopyrrolidin-1-yl) butanamide. Molecular formula is $C_8H_{14}N_2O_2$ and molecular weight is 170.20896. It is white or off white powder bitter in taste and soluble in water **[2]**.

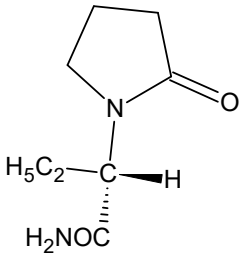

Figure 1: Chemical structure of levetiracetam

Exact mechanism of action of Levetiracetam is not known but it proposed that it binds to brain cell membranes in the CNS in saturable, reversible and stereo selective manner **[3]**. Blood concentration of levetiracetam was measured by fused silica capillary gas chromatography and nitrogen phosphorus detection by isocratic HPLC with UV detection **[4]**. It is completely absorbed by oral administration of dose 250-5000mg **[5]**. Volume of distribution is 0.5-0.7 L/kg. Tissue distribution data in human are not available but in rat it readily crosses the blood-brain barrier **[6]**. It is metabolized minimum that is after 24 hours 93 % administered dose is excreted **[7]**. Radio labeled studies of metabolism shows one major inactive acidic metabolite and two minor inactive metabolites. Its effect on cytochrome P450 was studied by *in- vitro* human liver microsomal markers **[8]**. CYP isoenzymes plays role in oxidative metabolism of endogenous and exogenous contents **[9]**. It is excreted in urine. Sixty-six percent drug eliminated unchanged and twenty four percent in urine as inactive metabolite. Fecal excretion is only 0.3% **[10]**.

Various qualitative and quantitative methods are used in bulk and pharmaceutical dosage form **[11-15]**. By ICH guidelines methods are developed and validated. Our goal of study was to develop simple and suitable HPLC method of levetiracetam.

MATERIAL AND METHODS

Levetiracetam pure drug was obtained as gift sample by Yarrow Chem Products, Mumbai. Marketed products Levipil 250 (Sun Pharmaceutical Industries Ltd), Levepsy 250 (Cipla Ltd) and Keppra 250 (UCB India Pvt Ltd) were used. HPLC (Agilents 1260 infinity II) was used. HPLC graded acetonitrile (Merck Specialities Pvt Ltd), water (Merck MilliQ) and aqueous o-phosphoric acid (Rankem distributors, Thane) was used.

Instrumentation

Selection of wavelength [16, 17]

Dilutions of levetiracetam (30µg/ml) were prepared. The λ max was

determined on UV – visible spectrophotometer (LMSPUV-1900) through the range 200 – 400nm **(Table 1)**.

Selection of mobile phase [18, 19]

A number of trials were performed to find out perfect mobile phase as shown in **Table 2**. The solvents such as water, acetonitrile was found. So the water and acetonitrile was mixed up and used for further chromatographic method.

Table 1: Optimized chromatographic conditions

1	Chromatograph	HPLC
2	Column Oven Temp	30°C
3	Flow rate:	1 ml/min.
4	Mobile Phase:	Water : Acetonitrile(50 : 50)
5	Runtime:	10 minutes
6	Injection Volume:	10µl
7	Wavelength:	205 nm
8	Diluent:	Water : Acetonitrile (50 : 50)
9	Column	Agilent Zorbax SB-Aq (250 x 4.6 mm, 5µ)

Table 2: Trials for selection of mobile phase

Sr. No.	MP	Ratio	Column	Flow rate	Diluents	Wavelength	RT	TP	Asymmetry
1	ACN: Water	50-50	Agilent Zorbax SB-Aq (250 x 4.6 mm, 5µ)	1 ml/min	50 ACN: 50 Water	205	2.58	9009	0.97
2	ACN: Water	45-55	Agilent Zorbax SB-Aq (250 x 4.6 mm, 5µ)	1 ml/min	50 ACN: 50 Water	205	2.63	10117	0.00
3	ACN: Water	55-45	Agilent Zorbax SB-Aq (250 x 4.6 mm, 5µ)	1 ml/min	50 ACN: 50 Water	205	2.53	10338	0.00
4	ACN: Water	50-50	Agilent Zorbax Bonus RP (250 x 4.6 mm, 5µ)	1 ml/min	50 ACN: 50 Water	205	2.37	9364	0.00
5	ACN: Water	40-60	Agilent Zorbax Bonus RP (250 x 4.6 mm, 5µ)	1 ml/min	50 ACN: 50 Water	205	2.46	5011	1.91

Preparation of Standard Solution

Initially Prepare a Standard Stock Solution (SSS-I) of Levetiracetam by adding 5mg in 10 ml volumetric flask & add 5 ml diluent. Mix and sonicate for 5 minutes. Make up the volume to 10 ml with diluent. (Conc. = 500µg/ml).

Working Standard (WS):

Pipette out 0.5 ml of SSS-I in 10 ml volumetric flask. Add 5 ml diluent, Sonicate and make up the volume with diluent. And Label it as WS. (Conc.25µg/ml)

Drug Product Sample (DPS) Preparation [20]

Weigh accurately 10 tablets of Keppra and crush in mortar and pestle. Weigh tablet powder equivalent to 5 mg of Levetiracetam in 10 ml volumetric flask. Add 5 ml diluents and sonicate for 10 min; make up the volume with diluents (Conc. = 500µg/ml). Pipette out 0.5 ml of above solution in 10 ml volumetric flask. Then add 5 ml diluents and mix for 10 min; make up the volume with diluents (Conc. = 25µg/ml).

Method Validation:

Specificity& Assay: Blank was injected to observe if any diluent peak is interfering

with the main peak and assay was also calculated.

$$\% \textbf{ Assay} = \frac{\textbf{Sample Area}}{\textbf{Standard Area}} \times \textbf{100}$$

Repeatability & System Suitability [21]

A single drug Product was prepared as described and 5 injections were made from same drug Product; checked for RSD and for system suitability. System suitability parameters are as below:

1. Retention time,
2. Theoretical plates,
3. Asymmetry (Tailing factor)

Linearity & Range [22, 23]

5 Keppra tablet samples of varying concentrations ranging from 60-140% were made. The concentrations are given in **Table 3**.

Accuracy [24]

The accuracy was determined by standard addition method. Three different levels (80%, 100% and 120%) of standards were spiked to commercial tablet. The mean of percentage recoveries and the % RSD was calculated **(Table 4)**.

LOD/ LOQ [25]

Limit of detection (LOD) and Limit of quantification (LOQ) can be calculated by using ANOVA Technique.

Table 3: % concentration

% Level	Levetiracetam Conc. (µg/ml)
60	15
80	20
100	25
120	30
140	35

Table 4: Preparation of sample

% Level	Levetiracetam concentration	Drug Product sample	Make up volume
60	15µg/ml	0.6 ml	10 ml
80	20µg/ml	0.8 ml	10 ml
100	25µg/ml	1 ml	10 ml
120	30µg/ml	1.2 ml	10 ml
140	35µg/ml	1.4 ml	10 ml

$$\boldsymbol{LOD} = \frac{\textbf{3.3} \times \boldsymbol{Std\ Error\ of\ Intercept}}{\boldsymbol{Coefficient\ of\ X\ variable\ 1}}$$

$$\boldsymbol{LOQ} = \frac{\textbf{10} \times \boldsymbol{Std\ Error\ of\ Intercept}}{\boldsymbol{Coefficient\ of\ X\ variable\ 1}}$$

RESULT AND DISCUSSION

Analytical wavelength:

The λ max observed at the wavelength for levetiracetam is 205nm. The absorption spectra of Levetiracetam are shown in **Figure 2**.

Linearity and Range:

A 50:50 mixture of acetonitrile and water was used and dilutions were made from Range 20-30 µg/ml for levetiracetam. The calibration graph was plotted with concentration of the drug against the peak area was found to be linear in the range of 20-3030 µg/ml. It obeys Beer's law over the concentration range. The RT at 100% was found 2.59. The regression equation and the correlation coefficient were found as in **Figure 3**.

LOD and LOQ:

The Limit of Detection and Limit of Quantitation for levetiracetam was found to be 2.20µg/ml and 6.66 µg/ml respectively **(Table 7)**.

Specificity:

The specificity of the method was found out by analyzing standard drug and Drug product (Keppra). The blank shows no peak and the Drug Product shows the RT 2.57 **(Figure 5, 6)**.

Accuracy:

The recovery study is done to judge the accuracy of the method. The study was carried out by adding a known quantity of pure drug to the formulation and proposed method was followed. The accuracy of method was determined by recovery study, percent recovery was calculated **(Table 9, 10)**.

Precision (Repeatability)

The precision of assay was determined by repeatability and %RSD was reported. The % RSD values obtained from peak area for LEV was observed **(Table 11)**.

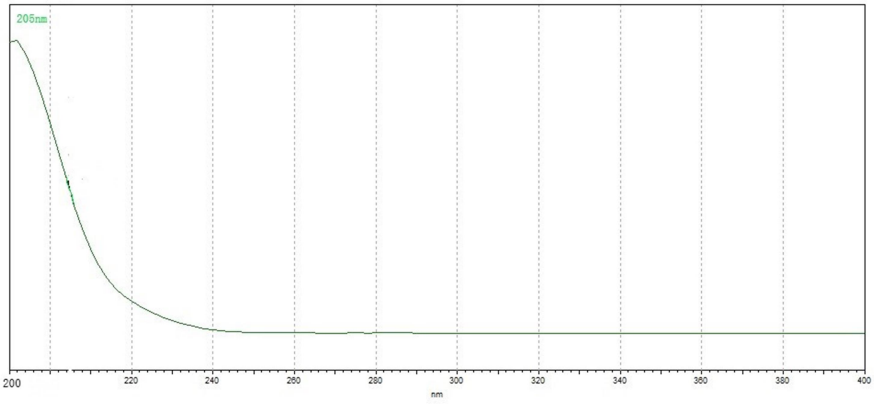

Figure 2: λ Max of levetiracetam

Table 5: Calibration curve data of levetiracetam

% Level	Concentration (µg/ml)	Area
80	20	1239115
90	22.5	1395183
100	25	1551314
110	27.5	1685445
120	30	1871577

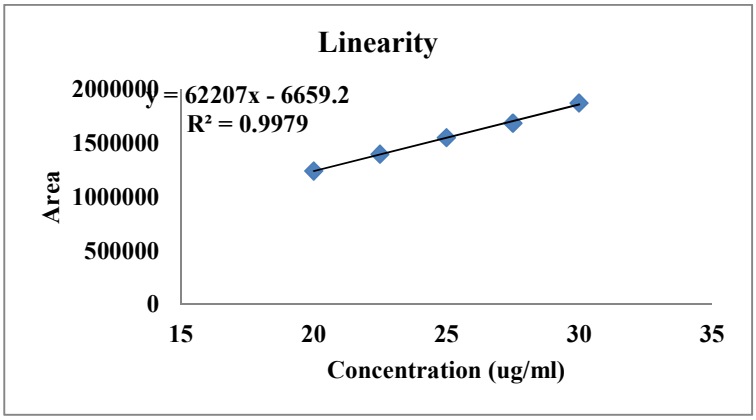

Figure 3: Calibration curve of levetiracetam

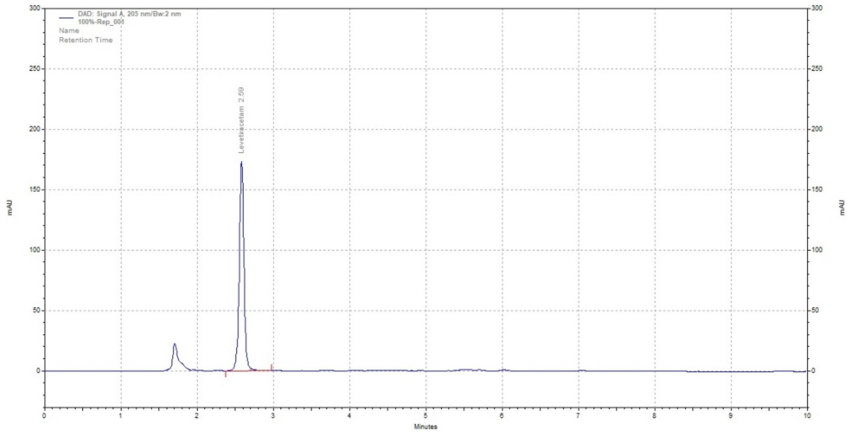

Figure 4: Typical Chromatogram of levetiracetam

Table 6: Regression Statistics

Regression Statistics	
Multiple R	0.998956761
R Square	0.99791461
Adjusted R Square	0.99721948
Standard Error	12979.8204
Observations	5

Table 7: LOD and LOQ by ANOVA

	Df	SS	MS	F	Significance F
Regression	1	2.4186E+11	2.42E+11	1435.579703	4.04429E-05
Residual	3	505427213.2	1.68E+08		
Total	4	2.42366E+11			

Table 8: LOD and LOQ Data

	Coefficients	Standard Error	t Stat	P-value
Intercept	-6659.2	41454.22204	-0.16064	0.882584781
X Variable 1	62207.44	1641.831844	37.88904	4.04429E-05

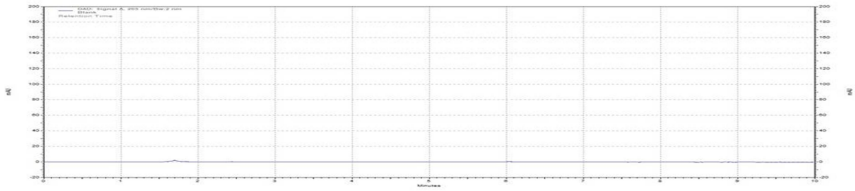

Fig. 5: Chromatogram for Blank

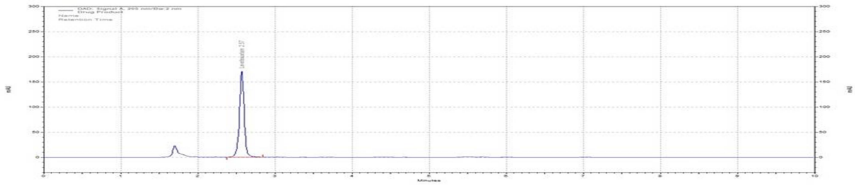

Fig. 6: Chromatogram for Drug Product

Table 9: Accuracy Data

STD wt. (mg)	Purity (%)	Potency (ug/ml)
5	99.7	498.5

Table 10: Accuracy Result

Sample ID	Reps	Spiked Conc. (ug/ml)	Area	Amt Recovered (ug/ml)	% Recovery	Average	STDEV	RSD
80%	Rep 1	19.94	1239115	19.90	99.79	99.81	0.022892	0.02
	Rep 2	19.94	1239517	19.90	99.82			
100%	Rep 1	24.925	1551314	24.91	99.95	99.95	0.00164	0.00
	Rep 2	24.925	1551350	24.91	99.95			
120%	Rep 1	29.91	1871577	30.05	100.48	100.47	0.012718	0.01
	Rep 2	29.91	1871242	30.05	100.46			

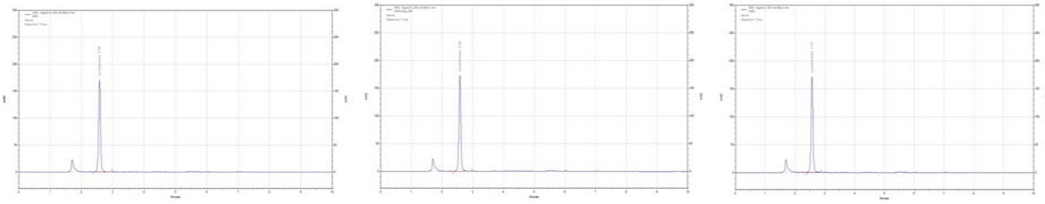

Fig. 7: 80% , 100 % and 120% Rep 1

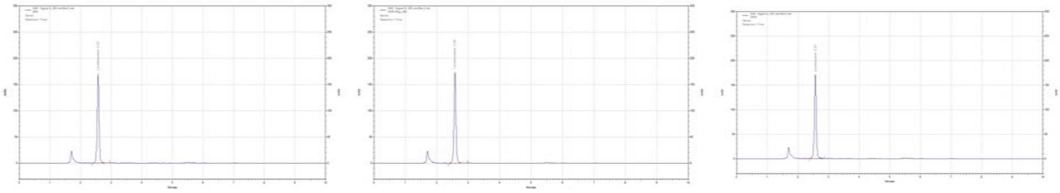

Fig. 8 : 80% , 100 % and 120% Rep 2

Table 11: Repeatability results

Sample ID	Area
Rep 1	1551314
Rep 2	1551350
Rep 3	1554594
Rep 4	1553332
Rep 5	1550203
Average	1552159
STDEV	1767.738
RSD	0.11

Table 12: System suitability parameters

Sample ID	RT	TP	Asymmetry
Rep 1	2.59	9173	0.97
Rep 2	2.58	9148	1.01
Rep 3	2.58	9153	1.01
Rep 4	2.58	9144	0.98
Rep 5	2.58	9156	0.99
Average	2.582		
STDEV	0.00447		
RSD	0.17		

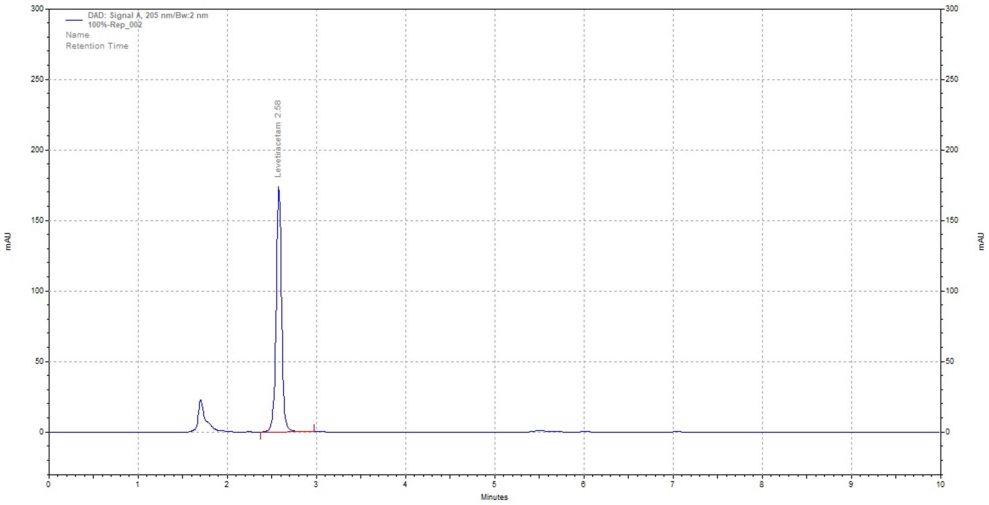

Figure 9: Chromatogram for Repeatability

By applying the proposed [RP-HPLC using DAD] method, the run time of method was set at l0 minutes and the levetiracetam appeared on typical chromatogram at 2.59 min as shown in **Figure 4**, which indicates good base line. To optimize the chromatographic conditions, various combinations of acetonitrile and water were tested as in **Table 2**. The use of acetonitrile and water in the ratio of 50:50 resulted in peak with good shape & resolution. Linearity range was observed in concentration range 20-30 µg/ml. The regression coefficient of Levetiracetam concentration was found to be y= 62207x- 6659. (r^2 0.997) where y is peak area and x is concentration of Levetiracetam (µg/ml) as shown in **Figure 3**. The Limit of Detection and Limit of Quantitation was found to be 2.20µg/ml and 6.66µg/ml respectively. The specificity for the developed RP-HPLC Method shows no peak for blank and for Drug product (Keppra 250) shows RT 2.57 as in **Figure 5** and **Figure 6** respectively. In the accuracy study of developed method the percentage recovery of levetiracetam ranging from 99.81-100.47 as shown in **Table 10** indicates that the proposed method is accurate. In case of Precision the Relative Standard Deviation in the peak area of drug for 5 replicate injections was found to be

less than 1% as in **Table 11**. The system suitability parameters for the developed method found as, the average asymmetry factor found to be 0.992 which indicates asymmetric nature of peak. The average number of Theoretical plates was found to be 9154 which indicates efficient performance of the Column, as resulted in **Table 12**. Hence, the developed RP-HPLC method was reliable, linear, accurate, specific method.

CONCLUSION

In above work done the suitable method for RP-HPLC Using DAD was developed and validated as per ICH Guidelines for determination of Levetiracetam in Bulk and from its formulation that is Keppra 250. From above study it was clear that the proposed method was simple, reliable, linear, repeatable, and cost effective with good precision & accuracy. The results obtained were in good agreement with the label Claim. Hence, this proposed method of RP-HPLC can be used for the routine determination of Levetiracetam in bulk & from its formulation.

REFERENCES

[1] Patsalos PN, Sander JWAS. Newer antiepileptic drugs. Towards an improved risk-benefit ratio. Drug Saf 1994 Jul; II: 37-67

[2] De Smedt T, Raedt R, Vonck K, Boon P. Levetiracetam: part II, the clinical profile of a novel anticonvulsant drug. CNS drug reviews. 2007 Mar; 13(1): 57-78.

[3] Noyer M, Gillard M, Matagne A, Hénichart JP, Wülfert E. The novel antiepileptic drug levetiracetam (ucb L059) appears to act via a specific binding site in CNS membranes. European journal of pharmacology. 1995 Nov 14; 286(2): 137-46.

[4] Ratnaraj N, Doheny HC, Patsalos PN. A micromethod for the determination of the new antiepileptic drug levetiracetam (ucb LO59) in serum or plasma by high performance liquid chromatography. Therapeutic drug monitoring. 1996 Apr 1; 18(2): 154-7.

[5] Browne TR. Pharmacokinetics of antiepileptic drugs. Neurology. 1998 Nov 1;51(5 Suppl 4): S2-7.

[6] Doheny HC, Ratnaraj N, Whittington MA, Jefferys JG, Patsalos PN. Blood and cerebrospinal fluid pharmacokinetics of the novel anticonvulsant levetiracetam (ucb L059) in the rat. Epilepsy research. 1999 Apr 1; 34(2-3): 161-8.

[7] Tong X, Patsalos P. Microdialysis study of levetiracetam in rat frontal cortex and hippocampus: 3.086.

Epilepsia. 1997; 38.

[8] Perucca E, Bialer M. The clinical pharmacokinetics of the newer antiepileptic drugs. Clinical pharmacokinetics. 1996 Jul; 31(1): 29-46.

[9] Rowland M. Clinical pharmacokinetics. Media, PA 19063-2043. 1995.

[10] Walker MC, Patsalos PN. Clinical pharmacokinetics of new antiepileptic drugs. Pharmacology & therapeutics. 1995 Jan 1; 67(3): 351-84.

[11] Valarmathy J, Samueljoshua L, Rathinavel G, Thanuja CS, Sivakumar T. RP-HPLC Method development and validation for assay of Levetiracetam in tablet dosage Form. Research Journal of Pharmacy and Technology. 2008; 1(4): 395-7.

[12] Hamdan II, Alsous M, Masri AT. Chromatographic characterization and method development for determination of levetiracetam in saliva: application to correlation with plasma levels. Journal of analytical methods in chemistry. 2017 Aug 7; 2017.

[13] Saravanan G, Jyothi G, Suresh Y, Annerao A, Ramakrishna M, Reddy MY, Ravibabu B. LC method for the determination of the stability of levetiracetam drug substance under stressing conditions. Chromatographia. 2008 Jan; 67(1): 173-7.

[14] Nikolaou P, Papoutsis I, Dona A, Spiliopoulou C, Athanaselis S. Development and validation of a GC/MS method for the simultaneous determination of levetiracetam and lamotrigine in whole blood. Journal of pharmaceutical and biomedical analysis. 2015 Jan 5; 102: 25-32.

[15] Siddiqui FA, Sher N, Shafi N, Wafa Sial A, Ahmad M, Naseem H. Development of new method for simultaneous analysis of piracetam and levetiracetam in pharmaceuticals and biological fluids: application in stability studies. Bio Med Research International. 2014 Jul 8; 2014.

[16] Center for Drug Evaluation and Research (1994) Reviewer Guidance; Validation of Chromatographic Methods ICH, Q1A (R2) Stability Testing of New Drug Substances and Products, Geneva.

[17] Debata J, Kumar S, Jha SK, Khan A. A New RP-HPLC method development and validation of dapagliflozin in bulk and tablet dosage form. Int J Drug Dev Res.

2017; 9(2): 48-51.

[18] Lacy CF, Armstrong LL, Goldman MP, Lance LL. Lexi-Comp's drug information handbook. Lexi-Comp, Canada. 2004; 13: 930-31.

[19] Momin MY, Yeole PG, Puranik MP, Wadher SJ. Reverse phase HPLC method for determination of aceclofenac and paracetamol in tablet dosage form. Indian journal of pharmaceutical sciences. 2006; 68(3).

[20] Bioanalytical Method Validation (2001) US Department of Health and Human Services, FDA, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM).

[21] Sanagapati M, Dhanalakshmi K, Nagarjunareddy G, Sreenivasa S. Development and validation of a RP-HPLC method for the estimation of dapagliflozin in API. International Journal of Pharmaceutical Sciences and Research. 2014 Dec 1; 5(12): 5394.

[22] Elder D. ICH Q6A Specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances. ICH Quality Guidelines: An Implementation Guide. 2017 Sep 27: 433-66.

[23] Green JM. Peer reviewed: a practical guide to analytical method validation. Analytical chemistry. 1996 May 1; 68(9): 305A-9A.

[24] Winslow PA, Meyer RF (1997) Defining a master plan for the validation of analytical methods. J Validation Technology. 1997; 14: 361-367.

[25] Jani BR, Shah KV, Kapupara PP. Development and validation of UV spectroscopic method for simultaneous estimation of dapagliflozin and metformin hydrochloride in synthetic mixture. International Journal of Research and Development in Pharmacy & Life Sciences. 2015 May 15; 4(3): 1569-76.