CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1200927

Available online at: http://www.iajps.com Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-UPLC METHOD FOR THE DETERMINATION OF RELATED SUBSTANCES IN LEVETIRACETAM DRUG SUBSTANCE

Tene Sivaganesh[1,2], K. Ramasrinivas[1], T. SivaRao[2*],U. SeshamRaju[1], Arun Mendiratta[1]

[1] Department **of** Analytical Chemistry, Aurobindo Pharma Ltd, Pydibhimavaram, India.

[2]Department of Inorganic & Analytical Chemistry, Andhra University, Visakhapatnam, 530003, India.

Abstract:

Stability-indicating RP-UPLC methods have been developed for determination and validation of related substances in Levetiracetam bulk drug samples. The method shows adequate separation of Levetiracetam from and their associated related substances and degradation products. An efficient Separation of Levetiracetam related substances and degraded products were achieved by using New Waters HSS T3 column with (100 mm x 2.1 mm, 1.8 µm) dimensions. The buffer Ortho phosphoric Acid at pH 2.0 and Acetonitrile initial gradient was taken in (95.0:5.0 v/v) with a flow rate of 0.5 ml/min and Wavelength at 210 nm. Run time of the method was found to be 6.0 min. The linear regression analysis data for calibration plots show good linear relationship with 0.999. The limit of detection and limit of quantification are determined for Levetiracetam chloramide, Levetiracetam acid and Levetiracetam 0.01%, 0.002%, 0.002% and 0.03%, 0.005%, 0.004% respectively. The signal to noise ratios was found more than 3.4 and 10.5 for all components determined in the method. Intra and Inter-day precision is less than 2.5%. The % recovery of Levetiracetam impurities are between 90.2 – 114.5 %. The drug is subjected to different stress conditions and the resulting degradation products obtained did not interfere with the detection of Levetiracetam.

Keywords: *Levetiracetam, Levetiracetam Chloramide, Levetiracetam Acid, RP-UPLC, Validation*

***Corresponding Author:**

T. SivaRao,

Department of Inorganic & Analytical Chemistry,
Andhra University,
Visakhapatnam, 530003, India.
Telephone: +91 7702110459,
Email: sivaraotvalluri.16@gmail.com

QR code

Please cite this article in press *T. SivaRao et al.,* ***Development and Validation of Stability Indicating RP-UPLC Method for the Determination of Related Substances in Levetiracetam Drug Substance,*** *Indo Am. J. P. Sci, 2018; 05(03).*

1.0. INTRODUCTION:

The development of new antiepileptic drugs for epilepsy over the last few years has been spur by the fact that the available antiepileptic drugs did not provide most advantageous care for patients with epilepsy [1]. Levetiracetam is a new antiepileptic drug that is currently used as an add-on therapy or monotherapy in patients with partial and secondary generalized seizures [2]. Levetiracetam has a broad spectrum in antiepileptic activity[3,4]. The efficacy of seizures treatment depends on the drug quality, which requires suitable monitoring. The quality controls of the anticonvulsant drugs are fundamental for the well-being of patients and are imperative for the development of routine analytical methods that can reliably measure these. Levetiracetam has been approved in the European Union as a monotherapy treatment for epilepsy in the case of partial seizures, or as an adjunctive therapy for partial, myoclonic and tonic-clonic seizures. It is also used in veterinary medicine for similar purposes. Levetiracetam has potential benefits for other psychiatric and neurologic conditions such as Tourette syndrome, autism, bipolar disorder and anxiety disorder. Side effects for this product are drowsiness, weakness, infection, loss of appetite, stuffy nose, tiredness and dizziness.

These side effects and problems occurred may be due to related impurities are degradation products. The structures of the Levetiracetam and its impurities are given bellow (Figure:1,2&3), (-)-(S)-α-ethyl-2-oxo-1-pyrrolidine acetamide,$C_8H_{14}N_2O_2$, molecular weight 170.21 gm/mol is chemically unrelated with other antiepileptic drugs in current use, differing in structure and pharmacology.

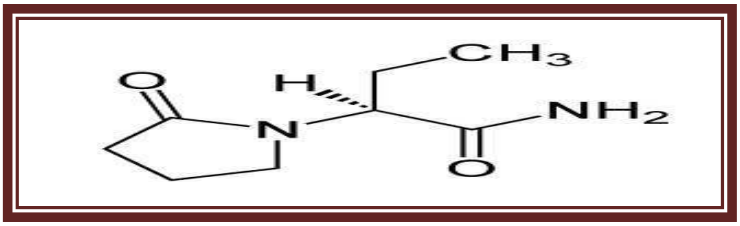
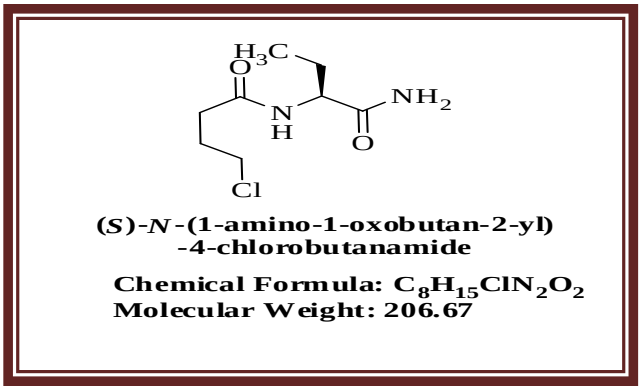


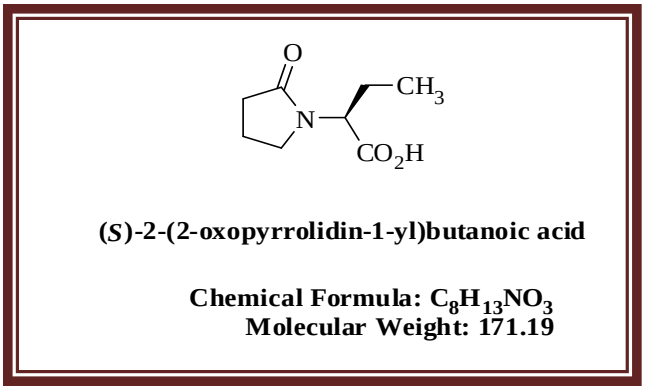
Figure: 1 Levetiracetam



(*S*)-*N*-(1-amino-1-oxobutan-2-yl) -4-chlorobutanamide

Chemical Formula: $C_8H_{15}ClN_2O_2$
Molecular Weight: 206.67

Fig. 2 Levetiracetam Chloramide



(*S*)-2-(2-oxopyrrolidin-1-yl)butanoic acid

Chemical Formula: $C_8H_{13}NO_3$
Molecular Weight: 171.19

Fig.3: Levetiracetam Acid

Table: 1

Product Name	Levetiracetam
Chemical Name	(S)-α-Ethyl-2-Oxo-1-Pyrrolidine Acetamide
CAS Reg.No:	(102767-28-2)
Molecular Formula	$C_8H_{14}N_2O_2$
Molecular Weight	170.21

The literature survey reveals that according to the HPLC methods for the determination of Levetiracetam in human plasma and API are reported (S.Gopalakrishnan et.al used Inertsil C18 column 250x 4.6mm; 5µm and this is only Assay method) [5], Rao BM et.al[6], (Basaveswara Rao M.V et al are used symmetry C18 (250 mm x 4.6 mm, 5µm) and the mobile phase used as Methanol:Acetonitrile) [7], (Ravisankar P et al are used symmetry C18 (250 mm x 4.6 mm, 5µm and this is only Assay method) [8], (Jignesh S. Shah et.al are used Phenomenex250 mm × 4.6 mm i.d., 5µm particle size and impurities are separated but not validated)[9],(Grace AC et.al are used Zorbax CN column 250×4.6 mm inner diameter, 5µm and 2 impurities are not identified) [10], (Abdullah Al Masud et.al are used Flexit C18 column 150 x 4.6 mm; 5µm but LOD and LOQ values are higher)[11], (M. Krishna Chaitanya Prasad et.al are used Phenomenex_ C18 (250 mm x 4.6 mm i.d, 5 µm particle size and impurities are separated but not validated)[12], (Lakshmana rao.A et.al are used RP-C18 column (250 mmx4.6 mm I.D; particle size 5 µm and monitored only main peak)[13], (Narendra Devanaboyina et.al are used Chromosil C18(250x4.6mm, 5µm in particle size and tailing is high, theoretical plates are low)[14] and (Poongothai.S et.al are used Prontosil C18-EPS, column150 mm x 4.6 mm i.d., with a particle size of 5 µm and Only Assay method) [15]. So all these literature reports indicated that the methods for detection, quantification and validation done by HPLC methods only .Very few literature reports are available for the determination and validation of Levetiracetam RP-UPLC methods reported by(Bahareh Mohammadi B.et.al are used Blue Orchid C18 1.8 µm, 50 × 2 mm this is extraction method LOD and LOQ values are higher) [16], (Nehal Fayek Farid et.al are used Hypersil Gold Cyanide column with dimensions of 15 cm × 2.1 mm, and particle size of 3 µm and one impurity only monitored)[17], (Parimal Patel et al are used Zorbax XDB C18 50 x 4.6 mm,1.8 µm separation of one impurity with the run time was 12 min) [18] and (E. Ola´h et.al are used BEH C18 column 1.7 mm particle size and 100 3 2.1 mm i.d. and only for Assay method) [19]. In all the HPLC methods the main drug were validated except related impurities but in the RP-UPLC method Assay and one impurity was validated. Based on the above reports there is no single method developed for simultaneous determination of 2 impurities[20,21] hence, in the present investigation we aimed to develop a method to simultaneous determination and validation of Levetiracetam along with 2 impurities by using RP-UPLC method with column Acquity UPLC HSS T3 100mm×2.1mm column with 1.8 µm partial size. Many scientists prefer reversed –phased chromatographic approaches to retain polar compounds.T3 particle technology is derived from C18 ligonds and proprietary end capping to achive optimum characteristics suited for polar compounds separation. Pore size plays a critical role to dramatically reduce retention time due to stationary phase hydrophobic collapse when using aqueous mobile phases. The Trifunctional T3 bonding and end-capping technology yields packing materials with superior low p^H stability.

2.0. EXPERIMENTAL:

2.1. Chemicals and reagents

Samples of Levetiracetam and its impurities were gifted from Aurobindo pharma Ltd, Pydibhimavaram, India. HPLC grade Acetonitrile, Methanol and AR grade Orthophosphoric Acid, KH_2PO_4 and Trifluroacetic acid were procured from Merck India Ltd, Mumbai, India. High pure water was prepared by using Millipore Milli-Q water purification system. Acquity UPLC HSS T3 100mm×2.1mm column with 1.8 µm partial size (Part no. # 186003539), Acquity UPLC HSS C18 100mm×2.1mm column with 1.8 µm partial size and Acquity CSH Phenyl hexyl (100 x 2.1 mm, 1.7 µm) was procured from Waters India Ltd, Bangalore, India. Among these 3 columns Acquity UPLC HSS T3 100mm×2.1mm column with 1.8 µm found to be efficiently separates the Levetiracetam and its 2 impurities. Hence HSS T3 100mm×2.1mm column with 1.8 µm was used method validation process.

2.2. Instrumentation

Total practical experiments, stress studies and validation studies were performed on Acquity-UPLC[22] system equipped with LC pump (model ACQ-BSM), an online degasser, auto sampler (model ACQ-SM) with thermostat, and a detector (TUV) (model ACQ-TUV). The data was acquired, monitored and processed using Empower3 software. Design expert® version 9 (Stat-Ease Inc., Minneapolis, USA) was used for optimizing the chromatographic conditions. The buffers p^H was monitored by using Metrohm 780 p^H meter and weights taken by using the Sartorius CPA225D balance.

2.3. Chromatographic conditions

The stationary phase used was Acquity UPLC HSS T3 100mm×2.1mm column with 1.8 µm partial size (Part no. # 186003539). Mobile phase contains Orthophosphoric Acid (A) whereas mobile phase contains Acetonitrile (B). The flow rate of the mobile phase is 0.5 mL/min. The gradient programme time (in min)/% mobile phase-B is set as 0/05, 2.2/15,

3.6/35, 4.3/35, 4.5/05, 6.0/05. The column temperature was maintained at 25°C and the absorption was monitored at 210nm. The injection volume was 2µL. Mobile phase-A used as a diluent. Based on parameters indicated that the run time was completed within 6 minutes compared to other chromatographic methods in the literature moreover the injection volume of the sample required for UPLC method is 2µL is sufficient but in the case of HPLC needs more than 10 -30µL.

Table 2: Gradient programmes

Time (min)	*Mobile phase A(% v/v)*	*Mobile phase B(% v/v)*
$T_{0.01}$	95	05
$T_{2.2}$	85	15
$T_{3.6}$	65	35
$T_{4.3}$	65	35
$T_{4.5}$	95	05
$T_{6.0}$	95	05

Table 3: Elution order:

S.No.	NAME	RRT	Response factor	Impurity Classification
1	Levetiracetam	= 1.00	--	--
2	Levetiracetam Chloramide	~ 1.35	6	Process
3	Levetiracetam Acid	~ 1.44	1.1	Process and Degraded

2.4. Preparation of standard solutions

System suitability solution: Weighed about 25 mg of Levetiracetam reference sample into a 50 mL clean, dry volumetric flask, added 30 mL of mobile phase A and sonicate to dissolve. Make up to volume with mobile phase A. Filtered through 0.22µ porosity membrane filter.

Standard solution: Weighed and transferred accurately 30 mg of Levetiracetam working standard into a 50 mL clean, dry volumetric flask, added 25 mL of mobile phase A and sonicate to dissolve. Make up to volume with mobile phase A. Diluted 5 mL of this solution to 50 mL with mobile phase A. Further diluted 5 mL of this solution to 100 mL with mobile phase A. Filtered through 0.22µ porosity membrane filter.

Sample solution: Weighed and transferred accurately 100 mg of sample into a 50 mL clean, dry volumetric flask, added 15 mL of mobile phase A and sonicate to dissolve. Make up to volume with mobile phase A. Filtered through 0.22µ porosity membrane filter.

2.5. Calculation of Levetiracetam related substances in API samples

The API samples, the percent of impurities were calculated below using equation

$$\frac{AT}{AS}\times\frac{WS}{50}\times\frac{5}{50}\times\frac{5}{100}\times\frac{50}{WT}\times P\times RF$$

Where AT is peak area due to Known/Unknown impurity in the sample preparation, AS is Average peak area due to Levetiracetam in the standard used for related substance and RF is relative response factor. WS is the weight of Levetiracetam standard taken in mg, WT is the Weight of the sample taken in mg, P is the Potency of the Levetiracetam working/reference standard.

3.0. RESULTS AND DISCUSION:

3.1. Method development:

Analytical method development and validation play important roles in the determination of drug substances and its related impurities in pharmaceutical products. The run time, injection volume, mobile phase consumption and requirement of all chemicals are very less by considering above aspects, UPLC instrument was selected for the method development.

3.2. Selection of stationary phase:

Based on the structure and molecular weight of API and impurities C18 columns like Waters BEH C18 column were initially screened for the separation. Among this columns failed to provide acceptable separation and peak shape. Several other C18 columns with other stationary phases were screened for separation but a remarkable selectivity was achieved with New Acquity UPLC HSS T3

100mm×2.1mm column with 1.8 µm partial size was finalised for the further optimization of chromatographic separation. This column was not used anywhere in another methods. This is new stationary phase.

3.3. Selection of Mobile phase

As one of the objectives of the method is to develop a different buffers ratios, orthophosphoric acid+water with p^H 2.0, trifluoro acetic acid+water, potassium dihydrogen orthophosphate +water buffers were evaluated. It is observed that in orthophosphoric acid buffer a promising candidate for separation of impurities. The organic modifiers, methanol and acetonitrile were evaluated at different gradients conditions. Based on the results, acetonitrile was finalised as organic modifier.

3.4. Selection of Diluent

Based on the solubility of Levetiracetam and its impurities, mobile phase-A was selected as diluent. It was also noticed that the sample and standard solutions prepared with this diluent were stable for at least 3 hrs at 25°C but these are stable for at least 15hrs at 5°C.

3.5. Experimental design for optimising flow rate, buffer concentration and column temperature

The initial method development trials with one factor at a time (OFAT) variation revealed that the flow rate and column temperature had significant impact on selectivity. It was observed that the critical resolution pairs for this method were resolution between Impurity-Acid, Impurity-chloramide. Since optimising the chromatographic parameters with OFAT approach consumes lot of time and does not provide the design space, a design of experiments (DoE) was used for optimising these chromatographic parameters. The design space defines the experimental region in which changes to method parameters will not significantly affect the quality and results. As working within the design space is not considered as a change, the scientist can have choice to operate the method at different chromatographic condition.

Based on the analysis, it is understood that, to obtain good resolution, column temperature and flow rate are maintained at 25°C and 0.5 mL/min respectively. The chromatographic conditions summarized in table: 4.

Table 4: Chromatographic conditions

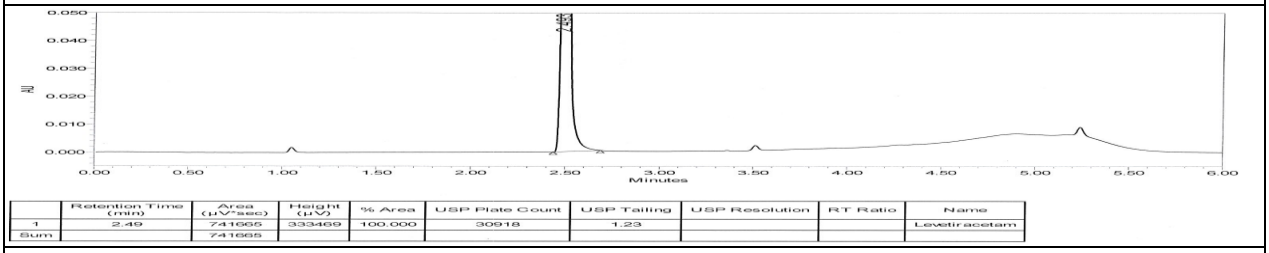
Instrument	:	UPLC make by Waters
Mode of analysis	:	Gradient
Flow rate	:	0.5 mL/min
Detector wave length	:	210 nm
Column temperature	:	25ºC
Injection volume	:	2.0 µL
Column	:	Acquity UPLC HSS-T3 (2.1X100mm, 1.8µ)
Run time	:	6.0 min
Sample Manager Temp	:	5°C

4. 0. METHOD VALIDATION

Method validation is a process by which it is established that the performance characteristics of the method met intended analytical application. The analytical method was validated as per the ICH Q2 (R1) guideline [23,24].

4.1. System suitability

In the optimized UPLC conditions, system suitability parameters were evaluated for Levetiracetam and its two impurities (Fig. 4). Tailing factor for Levetiracetam was not more than 2.0. The USP plate count for Levetiracetam is more than 10000. The results are summarised in Table: 5



	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing	USP Resolution	RT Ratio	Name
1	2.49	741665	333469	100.000	30918	1.23			Levetiracetam
Sum		741665							

Fig.4: System suitability chromatogram

Table: 5 System suitability parameters.

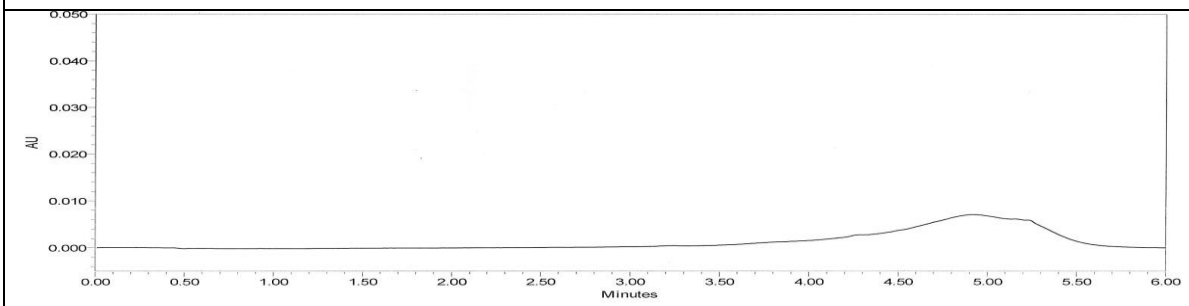
Parameters	Levetiracetam	Levetiracetam chloramide	Levetiracetam Acid
Tailing factor	1.23	NA	NA
Number of theoretical plates	30918	NA	NA
Retention time min	~2.49	~3.25	~3.41

4.2. SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, and matrix. Specificity was demonstrated by following three experiments.

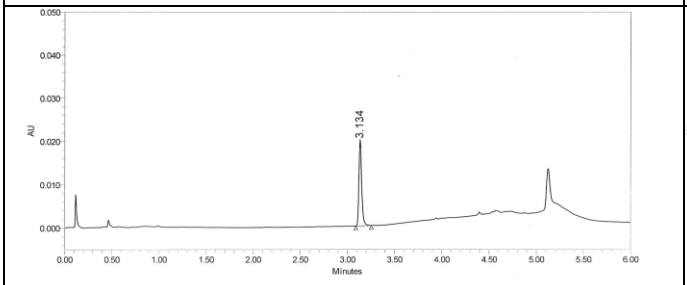
4.2.1. Blank and Impurity interference

The blank and individual impurities were prepared and injected in UPLC. No interference was observed at any of the peaks of interest in blank. The chromatograms are depicted in Fig: 5, 6 and 7



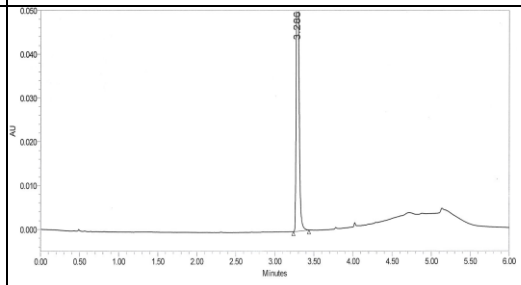
	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing	USP Resolution	RT Ratio	Name
1									
Sum									

Fig.5: Blank chromatogram



	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing	USP Resolution	RT Ratio	Name
1	3.13	40702	19955	100.000	57187	1.31			Levetiracetam Chloramide
Sum		40702							

Fig.6: Levetiracetam Chloramide



	Retention Time (min)	Area (µV*sec)	Height (µV)	% Area	USP Plate Count	USP Tailing	USP Resolution	RT Ratio	Name
1	3.29	202371	103009	100.000	70239	1.35			Levetiracetam Acid
Sum		202371							

Fig.7: Levetiracetam Acid

4.2.2. Forced degradation/ Stress study

The stress studies for Levetiracetam were performed at concentration 2mg/mL to provide an indication of the stability indicating property and specificity of the proposed method. The stress studies were performed on API samples, individual active drug substance to provide an indication and identification of the generated impurities of the drug substance. Intentional degradation was attempted with stress conditions of acid (5N HCl for 30 min), base (5N NaOH for 30min), oxidation (30% H2O2 for 30min), Photolytic (exposure to sunlight at window shade 48hours i.e. equal to watt hours/square meter and 1.2 million lux hours) and thermal (100 °C for 24hours) to evaluate the ability of the proposed method to separate the impurities of Levetiracetam from its degradation products. The results are summarised in table: 6. The chromatograms are depicted in Fig: 8-15

Impurity Mixture

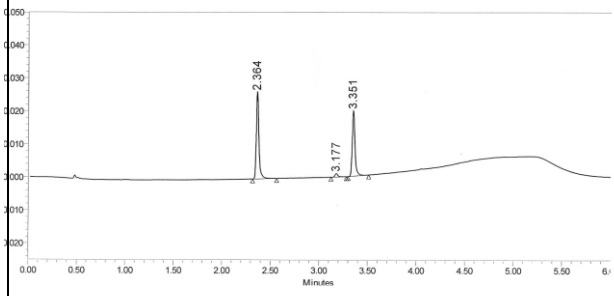


Fig.8: (Levetiracetam+Acid+Chloramide)

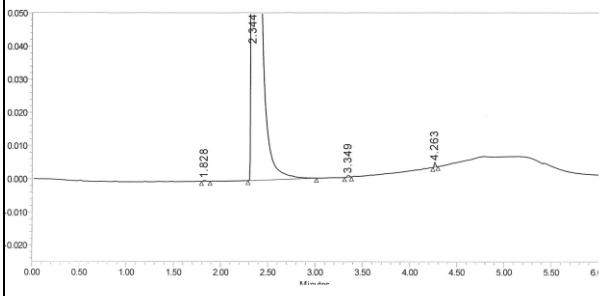


Fig.9: Undegraded Sample chromatogram

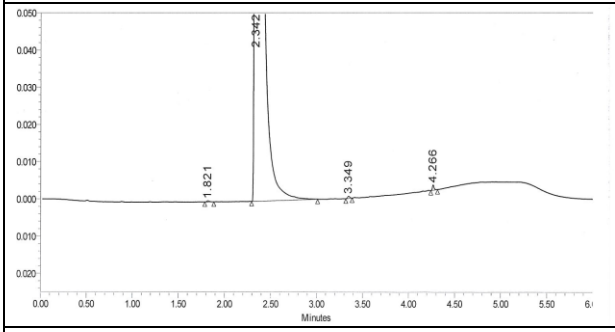


Fig.10: Sample chromatogram of Thermal degradation

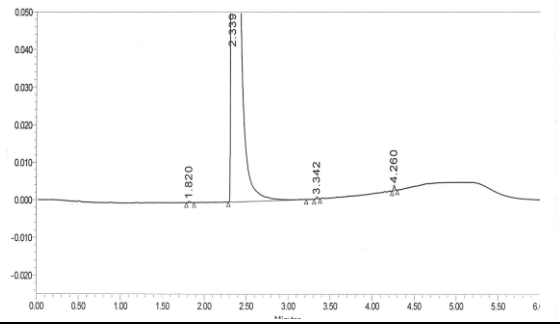


Fig.11:Sample chromatogram of Photo degradation

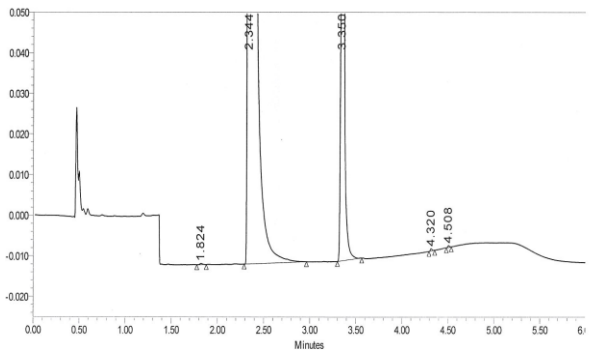


Fig.12: Sample chromatogram of Acid degradation

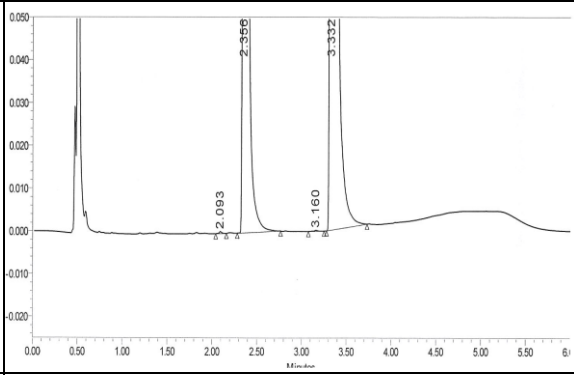


Fig.13: Sample chromatogram of Base degradation

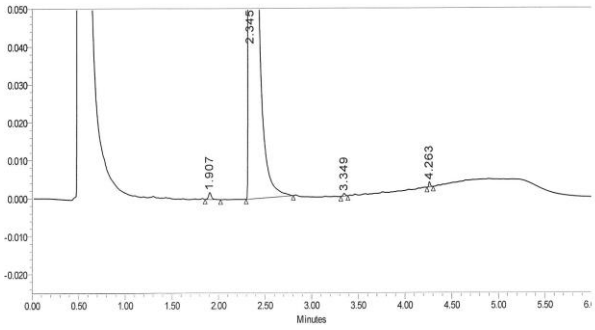


Fig.14: Sample chromatogram of Peroxide degradation

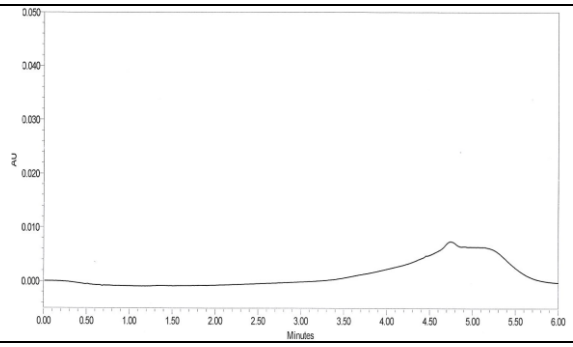


Fig.15: Blank chromatogram

Table 6: Degradation study

Degradation study						
S.No	Condition	Time	% Degradation			%Sample
			Acid	Chloramide	Unknown	
1	Undegraded sample	Fresh	0.012%	ND	0.02%	99.96%
2	5M HCL	30min	5.09%	ND	0.01%	94.88%
3	5M NaOH	30min	68.90%	0.01%	ND	31.08%
4	30% H2O2	30min	0.015%	ND	0.05%	99.92%
5	Thermal 100°C	24 hrs	0.012%	ND	0.021%	99.96%
6	Photo Degradation	48 hrs	0.013%	ND	0.02%	99.96%

4.3.LINEARITY:

4.3.1. Linearity for related substances of Levetiracetam:

To establish linearity of the related substance method, solutions were prepared by diluting the impurity stock solution to obtain the required concentrations at different levels ranging from 0.5% to 150%. The correlation coefficient, slope and y-intercept of the calibration curve were calculated. Further the relative response factors for all impurities were evaluated based on the S/N ratio method. The results are summarised in Table: 7. The chromatogram are depicted in Fig: 16

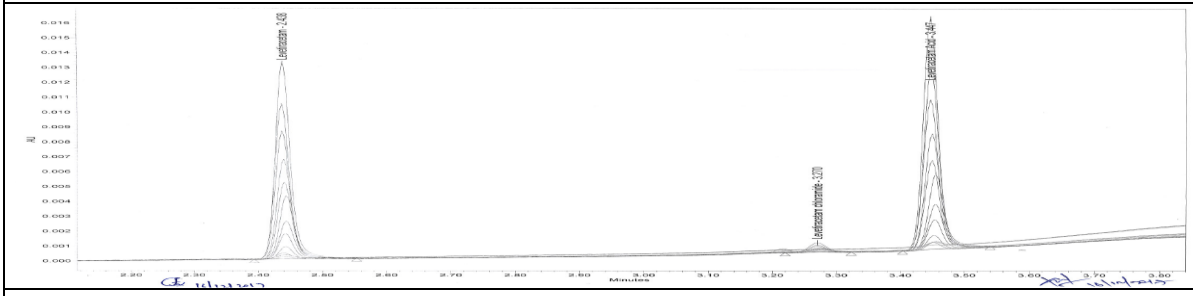


Fig.16: Levetiracetam +Levetiracetam chloramide+Levetiracetam Acid Linearity solutions

Table 7: Linearity (Correlation coefficient)

Linearity (Correlation coefficient)			
Levetiracetam	Levetiracetam chloramide	Levetiracetam Acid	Criteria for Correlation
0.9996	0.9928	0.9996	NLT 0.99

Inference: Based on correlation coefficient values from the table :7 two impurities and Levetiracetam were within the criteria.

4.4. LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The limit of detection (LOD) and limit of quantitation (LOQ) were established for Levetiracetam and its related compounds by diluting the standard stock solution. The % of LOD & LOQ of Levetiracetam chloramide, Levetiracetam Acid and Levetiracetam are calculated as (0.01%, 0.002%, 0.002 %,) and (0.03%, 0.005%, 0.004%) respectively. The signal to noise ratios was found to be more than 3.4 and 10.5 for all components. Hence these concentrations were finalised as LOD and LOQ concentrations. Further precision was found 7.21% at LOQ level for all impurities and the results are summarised in Table: 8

Table 8: Precision at LOD & LOQ of Levetiracetam chloramide, Levetiracetam Acid and Levetiracetam

Precision(LOD)			
Levetiracetam	Levetiracetam chloramide	Levetiracetam Acid	Criteria %RSD
6.68	11.58	8.90	NMT 33%
Precision(LOQ)			
Levetiracetam	Levetiracetam chloramide	Levetiracetam Acid	Criteria %RSD
5.20	7.21	3.37	NMT 10%

4.5. ACCURACY:

4.5.1. Accuracy for related substance of Levetiracetam

For the determination of accuracy of related substances method, recovery study was carried out by analysing the spiked samples. Known amounts of impurities were spiked in triplicate at three different concentration levels 50%, 100%, 120% to a previously analysed drug substance sample. The percentage of recoveries for two impurities was calculated. The results are summarised in below Table: 9.

4.6. PRECISION

The precision of the method was demonstrated by system precision and method precision.

4.6.1System precision:

System precision for related substances was demonstrated by injecting standard solution under the same operating conditions. The peak areas of Levetiracetam were measured and the % RSD was found to be 0.22%. The results are summarised in Table: 10. The chromatogram are depicted in Fig: 17

Table 9: Accuracy data (Analyte recovery study)

Accuracy data (Analyte recovery study)				
% of drug added	Spiked conc. (W/W)	Recovered conc. (W/W)	% Recovery	*% RSD
Levetiracetam Chloramide 50%	0.0258	0.0296	114.5	9.7
Levetiracetam Chloramide 100%	0.0503	0.0470	93.5	5.7
Levetiracetam Chloramide 120%	0.0626	0.0565	90.2	1.3
Levetiracetam Acid 50%	0.1015	0.1068	105.2	9.0
Levetiracetam Acid 100%	0.1974	0.1985	100.6	3.6
Levetiracetam Acid 120%	0.2458	0.2620	93.8	4.4
*Mean of three replicates				

Inference: Results from the table: 9, it is illustrated that recovered concentration of spiked Impurities were found to be within the acceptance criteria. i.e. 85% to 115%

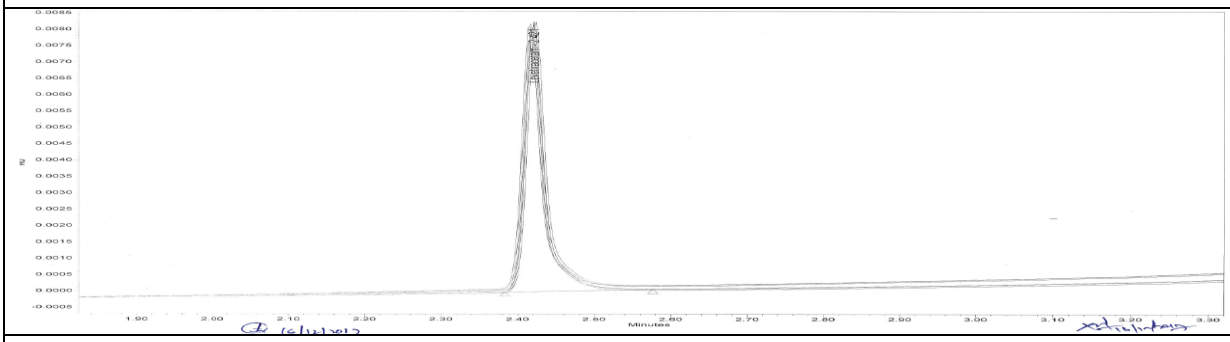


Fig.17: Levetiracetam

Table 10: System precision

System precision	
Levetiracetam	Criteria % RSD
0.22	NMT5

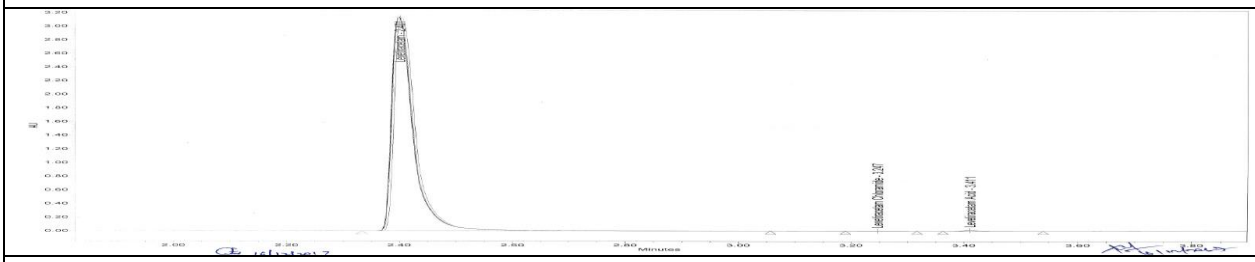


Fig.18: Levetiracetam +Levetiracetam chloramide+Levetiracetam Acid

Table 11: Method precision

Method precision			
Levetiracetam	Levetiracetam chloramide	Levetiracetam Acid	Criteria (% RSD)
0.14	2.11	0.16	NMT10

4.6.2. Method precision:

Method precision for related substances was illustrated by preparing six different samples by spiking two impurities at spec level. These solutions were injected along with a standard solution of Levetiracetam prepared at spec level. The areas corresponding to all impurities were measured and calculated the content of each impurity. The relative standard deviation of impurities obtained from all six preparations was found to be less than 2.11%. The results are summarised in Table: 11. The overlay chromatograms are depicted in Fig: 18

4.6.3. Intermediate precision (Ruggedness):

Intermediate precision for related substance was demonstrated by preparing six different samples by spiking two impurities at spec level by different analyst and different day. These solutions were injected along with a standard solution of Levetiracetam prepared at spec level. The areas corresponding to all impurities were measured and calculated the content of each impurity. The relative standard deviation of impurity content obtained from all six preparations was found to be less than 2.25%. The results are summarised in Table: 12. The overlay chromatograms are depicted in Fig: 19.

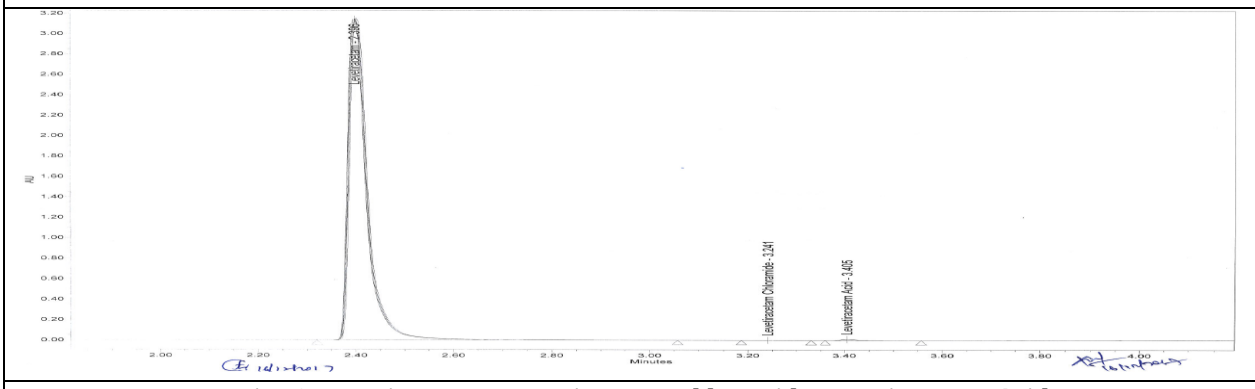


Fig.19: Levetiracetam +Levetiracetam chloramide+Levetiracetam Acid

Table 12: Intermediate precision

Intermediate precision			
Levetiracetam	Levetiracetam chloramide	Levetiracetam Acid	Criteria (% RSD)
0.42	2.25	0.31	NMT10

Table 13: Cumulative RSD for precision

Cumulative RSD for precision			
Levetiracetam	Levetiracetam chloramide	Levetiracetam Acid	Criteria (% RSD)
0.33	2.49	1.53	NMT15

Inference: Results from table 11,12 and 13 it is observed that cumulative RSD's were found to be within the limit(i.e. NMT 15%)

Table 14: HPLC Vs UPLC results Comparison report

HPLC Vs UPLC results Comparison report							
Details		Chloramide		Acid		Unknown	
S.No	B.No	HPLC	UPLC	HPLC	UPLC	HPLC	UPLC
1	Sample-01	0.001	0.001	0.015	0.015	0.015	0.015
2	Sample-02	0.001	0.001	0.010	0.009	0.014	0.013
3	Sample-03	0.002	0.001	0.011	0.010	0.014	0.015
4	Sample-04	ND	ND	0.019	0.017	0.054	0.056
5	Sample-05	ND	ND	0.017	0.016	0.055	0.054
6	Sample-06	ND	ND	0.015	0.016	0.038	0.039
7	Sample-07	0.001	0.002	0.019	0.019	0.019	0.019
8	Sample-08	0.001	0.002	0.019	0.018	0.018	0.019
9	Sample-09	0.001	0.001	0.021	0.022	0.018	0.017
10	Sample-10	0.001	0.002	0.01	0.01	0.017	0.018
11	Sample-11	0.001	0.001	0.01	0.01	0.019	0.015
12	Sample-12	ND	ND	0.014	0.012	0.022	0.016

Inference: Based on the above data results comparable to HPLC and UPLC each other.

4.7. FILTER COMPATIBILITY:

Filter compatibility to the sample is concluded from the recovery study indicated that there is no absorption of these components to filter.

4.8. SOLUTION STABILITY:

The standard and samples solutions were kept in refrigerator at 5°c and injected the aged samples (every 1hour) into the UPLC. The peak area corresponding to Levetiracetam and all impurities were measured. Calculated the similarity factor and found that the values are below 10% RSD. Thus indicates the sample and standard solutions are stable for at least 15hrs when stored on refrigerator condition.

4.9. METHOD ROBUSTNESS:

The critical method parameters like flow rate of mobile phase, column temperature, wavelength and buffer concentration were deliberately varied and found that the method was robust from flow rate 0.45– 0.55mL/min, buffer Ph 1.9 to 2.1,wavelength 208 to 212nm and Column temperature 23°C to 27°C. As mobile phases A and B were not a mixture of solvents, study of the mobile phase composition was not required. Based on this, the method is proved to be robust and can easily be implemented in quality control laboratories for the regular analysis of Levetiracetam samples with great confidence.

5.0 BATCH ANALYSIS:

Based on method development and validation analysed different streams batches analysis with final chromatographic conditions by UPLC. The results comparisons of HPLC Vs UPLC are summarized in table 14.

6.0. DISCUSSION:

The purpose of the present work was to develop a short, robust, UPLC method for the accurate quantitation of Levetiracetam and its specified impurities mentioned in the European Pharmacopeia and United States pharmacopeia. This method is developed for drug substance. As mentioned in the introduction section, several reports are available for quantification of Levetiracetam drug alone but limited literature is available on the separation of Levetiracetam and its specified impurities. After performing some initial experiment, through review of stationary phases, for the method development, optimisation and validation. Due to its diverse material attributes, significant selectivity was achieved among all impurities. During the development, flow rate and temperature was found to be critical chromatographic parameters. Hence, DoE was employed to understand the effect of these parameters on the selectivity. DoE has helped in finalising the flow rate and column temperature. The developed method was successfully validated for drug substance as per the ICH guideline. The proposed method is much superior to reported methods in terms of solvent consumption, run time, instrumental technique (UPLC), selectivity, and applicability to impurities analysis, applicability to drug substance and drug product. Different batches

analysis also conducted by using the optimised chromatographic conditions it's found satisfactory.

7.0. APPLICATION TO PHARMACEUTICAL INDUSTRY

This work will help industry to develop, manufacture and launch the product in a quick and economical way which in turn reduces the cost of the medicine and help the patient to avail quality, innovative and affordable medicines.

8.0. CONCLUSION:

A stability indicating RP-UPLC method has been developed for simultaneous identification of Levetiracetam along with the Determination of two related compounds. Developed method is proved to be robust using the experimental design, this method can successfully implemented in the quality control lab for the routine analysis of this product. Further this UPLC method was successfully validated as per ICHQ2 (R1) guideline and proved to be precise, linear, sensitive, accurate, and robust. This method is short and simple; hence implementation of this method in quality control and analytical development labs can give good results. As lesser amounts of solvents are required, implementation of this method will be environment friendly. This is the first RP-UPLC method that can separate and accurately quantitative Levetiracetam and two known impurities.

ACKNOWLEDGEMENTS:

Authors acknowledge Aurobindo Pharma Ltd and Andhra University for providing lab facility and required guidance to complete the work.

REFERENCES:

1.Fuchs FD, Wannmacher L. FarmacologiaClínica. Rio de Janeiro, Brazil: Guanabara Koogan; 2004.
2. Joel GH, Lee EL, Alfred GG. Goodman & Gilman's The Pharmacological Basis of Therapeutics, 10/e, Ed.Brazil: McGrawHill, 2003.
3.Menachem EB. Current Opinion in Neurology, 2000, 13(2), 165-170.
4.Abou-Khalil B. Neuropsychiatr, Dis Treat, 2008, 4 (3), 507–23.
5.S.Gopalakrishnan, B. Jeyashree, A. Ramalakshmi and A. Chenthilnathan Der Pharmacia Lettre, 2011, 3(3): 194-201
6.Rao BM, Ravi R, Shyam SR, Siva Kumar B, Gopichand S. J pharm Biomed Anal, 2004, 35 (5), 1017-26.
7.Basaveswara Rao MV et al. / Journal of Pharmacy Research 2012,5(1),75-78
8. Ravisankar P et al.Int.Res.J.Pharma.2015,6(8)
9.Jignesh S. Shah, G. Vidyasagar and H. Barot. Der Pharmacia Sinica, 2012, 3 (5):576-589
10.Grace AC, Prabha T, Jagadeeswaran M, Srinivasan K, Sivakumar T (2016) Simultaneous Determination of Levetiracetam and its Preservatives In Oral Liquid Dosage form by Rp-Hplc Method. SOJ Mater Sci Eng 4(2): 1-5.
11.Abdullah Al Masud1, Md. Shamsul Arefeen, Mohammad Kaisarul Islam, Moynul Hasan J. Chem. Pharm. Res., 2011, 3(1):324-329
12.Krishna Chaitanya Prasad.M, Vidhya Sagar G., Sudhakar P.Indian J.Pharm.Biol.Res. 2013; 1(4):45-54
13.Lakshmana rao A.and Naga jahnavi .V.E-Journal of Chemistry 2010, 7(2), 600-604
14.Narendra Devanaboyina, Satyanarayana T and Ganga Rao B. Der Pharma Chemica, 2011, 3 (6):112-117
15.Poongothai S, Balaji V, Madhavi B, Rajasekhar Reddy A, Ilavarasan R. and Karrunakaran C.M. International Journal of PharmTech Research Vol. 3, No.2, pp 1023-1032
16.Bahareh Mohammadi, Mohammadbagher Majnooni, Nazanin-sadat Afnanzade, Ronak jalili and Gholamreza Bahrami.African Journal of Pharmacy and Pharmacology Vol. 6(27), pp. 2017-2022, 22 July, 2012
17.Nehal Fayek Farid and Nada Sayed Abdelwahab European Journal of Chemistry 7 (3) (2016) 329-333
18.Parimal Patel et al. / Journal of Pharmacy Research 2011,4(12),4495-4497
19.Ola´h E, Bacso Gy, Fekete J and Sharma V.K Journal of Chromatographic Science,2012;50:253–258
20.http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3A_R2/Step4/Q3A_R2Guideline.pdf (accessed on April 21, 2016).
21.http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3B_R2/Step4/Q3B_R2Guideline.pdf (accessed on April 21, 2016).
22.Michael E. S., UPLC: An Introduction and Review; Journal of Liquid Chromatography & Related Technologies, (2005); 28:1253-1263.
23.http://www.fda.gov/downloads/Drugs/.../Guidances/ucm073507.pdf(accessed on April 21, 2016).
24.http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf (accessed on April 21, 2016).