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*Original Research Article*

# Stability indicating RP HPLC method for determination of levitiracetam in pharmaceutical formulation

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**ABSTRACT**

The article reports on a development of RP-HPLC method for the quantitative determination of Levetiracetam in tablet dosage forms. The chromatographic separations were performed using Phenomenex® C18 (250 mm x 4.6 mm i.d, 5 µm particle size) column at 40 ºC temperatures. The optimum mobile phase consisted of methanol, water and acetonitrile in the ratio of 30:10:60. Auto sampler 20 µl was used and kept at 15 ºC temperature. Analysis was done with flow rate of 1.0 ml/min at 212 nm (λ max of Levetiracetam) wavelength by using photodiode array (PDA) detector. The drug was analyzed for acid, alkaline, oxidative, hydrolytic, photolytic and thermal degradation studies. The standard calibration curve was plotted for the drug and results showed that the drug was linear ($r^2$ = 0.999) in the concentration range between 0.01 – 1.5 µg/ml. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the selected method is selective and stability-indicating for determination of levitiracetam in pharmaceutical formualtion.

## 1. Introduction

Levetiracetam is an analogue of piracetam which is used as an adjunct in the treatment of partial seizures with or without secondary generalizations in adults and childrens aged 4 years and over. It is also licensed for use as an adjunct in the treatment of primary generalized tonic-clonic seizures in adults and childrens with idiopathic generalized epilepsy [1]. A few analytical methods have been reported for the determination of Levetiracetam in pure drug, pharmaceutical dosage forms using spectrophotometry [2], liquid chromatography [3-10], gas chromatography[11], ion exchange chromatography [12], electrokinetic chromatography [13,14], capillary electro - chromatography[15],electrophoresis [16] and electrochemical method [17, 18]. The present study was aimed at developing a simple, sensitive, precise and accurate HPLC method for the stability analysis of Levetiracetam from tablet dosage forms.

## 2. Material and Methods

Levetiracetam standard were provided by Zydus Cadila Health Care, Ahmedabad, India. "Levexx - 250" tablet was procured from local market. Methanol was supplied by Merck Pvt. ltd, Mumbai. HPLC grade distilled water, Acetonitrile and all other

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reagents used in this study were of AR grade and were supplied by Merck Pvt. ltd, Mumbai.

### 2.1 *Instrumentation and chromatographic conditions:*

The chromatographic separations were performed using Phenomenex® C18 (250 mm x 4.6 mm i.d, 5 µm particle size) column at 40 ºC temperatures. The optimum mobile phase consisted of methanol, water and acetonitrile in the ratio of 30:10:60. Auto sampler 20 µl was used and kept at 15 ºC temperature. Analysis was done with flow rate of 1.0 ml/min at 212 nm (λ max of Levetiracetam) wavelength by using photodiode array (PDA) detector.

### 2.2 *Determination of wavelength of maximum absorbance*

The standard solution of Levetiracetam was scanned over wavelength of 200 to 400 nm by using UV-Visible spectrophotometer and 212 nm was selected as analytical wavelength for analysis of levetiracetam in pharmaceutical dosage form.

### 2.3 Preparation of mobile phase

HPLC grade solvents were used in separate bottle of gradient pumps as mobile phase. Methanol, water and acetonitrile in the ratio of 30:10:60 v / v were adjusted by gradient pump operated by LC solution software. Mixed solvents were degassed by the instrument and used as mobile phase.

### 2.4 *Preparation of standard stock solution (100 µg/ml)*

Accurately weighed 10 mg levetiracetam was transferred to 100 ml volumetric flask, dissolved in 50 ml methanol and diluted up to mark with methanol.

### 2.5*Preparation of standard working solution (10 µg/ml)*

Accurately measured 10 ml of standard stock solution was transferred to 100 ml volumetric flask and diluted up to the mark with methanol to obtain 10 µg/ml of levetiracetam.

### 2.6 *Preparation of calibration curves of levetiracetam*

Appropriate aliquots of standard working solutions of levetiracetam (0.01, 0.1, 0.2, 0.4, 0.6, 1.0 and 1.5 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with mobile phase. The solutions were filled in the vials of auto sampler rack. Aliquots (20 µl) of each solution were injected from auto sampler under the operating chromatographic conditions described above. Calibration curve was constructed by plotting peak area versus concentration of levetiracetam and the regression equation was calculated as depicted in Figure 1.

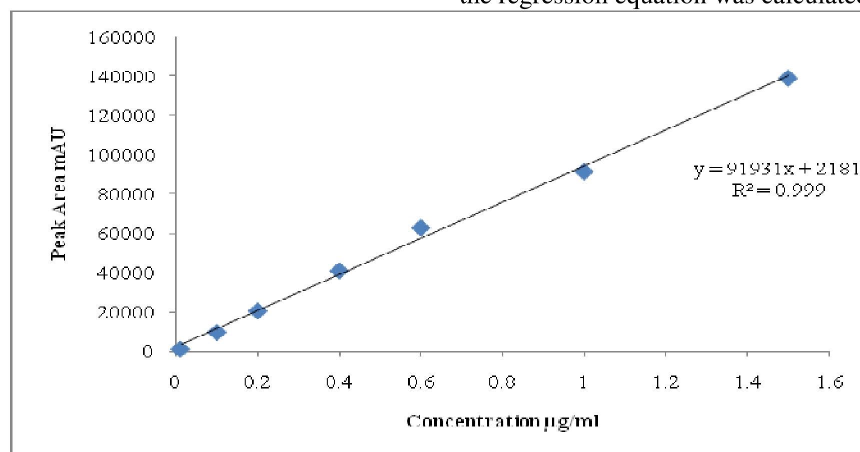


**Figure 1: Calibration curve of Levetiracetam at 212 nm by RP-HPLC method**

### 2.7 *Preparation of sample solution (10 µg/ml)*

Contents of twenty tablets "Levexx – 250" were accurately weighed and average weight per tablet was determined, contents were grounded to fine powder. An accurately weighed quantity of the pulverized powder equivalent to 10 mg of Levetiracetam was weighed and transferred to 100 ml volumetric flask, dissolved in methanol (60 ml) and sonicated for 30 min. The solution was filtered through Whatmann filter paper No. 41 and residue was washed with methanol. To ensure, the solution was filtered again through 0.45 µ filter paper. The solution was diluted up to the mark with methanol. Accurately measured 10 ml of standard sample stock solution was transferred to 100 ml volumetric flask, diluted up to the mark with methanol to get final working concentration of 10 µg/ml.

### 2.7 STABILITY STUDIES

#### I. *Force degradation study*

In order to determine whether the analytical method and assay were stability-indicating, levetiracetam tablet powder was stressed under various conditions to conduct forced degradation studies. As levetiracetam is freely soluble in methanol, so methanol was used as solvent in all studies. All solutions for forced degradation studies were prepared to yield starting concentration 100 µg/ml of Levetiracetam. In all cases, tablet powder contents equivalent to 10 mg Levetiracetam was

accurately weighed and prepared for analysis as previously described.

### II. *Acid degradation study*

Solution of levetiracetam (100 µg/ml) for acid degradation study was prepared using 0.1N hydrochloric acid in methanol and the resultant solution was refluxed at 60 ºC for 4 hours to facilitate acid degradation of levetiracetam.

### III. *Alkali degradation study*

Solution of levetiracetam (100 µg/ml) for alkali degradation study was prepared using 0.1N sodium hydroxide in methanol and the resultant solution was refluxed at 60 ºC for 4 hours to facilitate alkali degradation of levetiracetam.

### IV. *Wet heat (Hydrolysis) study*

Solution of levetiracetam (100 µg/ml) for wet heat degradation study was prepared using triple distilled water and the resultant solution was refluxed at 60 ºC for 4 hours to facilitate hydrolysis of levetiracetam.

### V. *Oxidation study*

Solution of levetiracetam (100 µg/ml) for oxidation study was prepared using 3 % $H_2O_2$ in methanol and the resultant solution was refluxed at 60 ºC for 8 hours to facilitate oxidation of levetiracetam.

### VI. *Dry heat study*

Solution for dry heat study were prepared by exposing approximately 50 mg of tablet powder in aluminium foil to dry heat in an oven at 60 ºC for 24 hour to facilitate dry heat degradation of levetiracetam. The tablet powder was removed from oven and the content of powder equivalent to 10 mg of levetiracetam was accurately weighed and transferred to volumetric flask and dissolved in methanol to make the final concentration 100 µg/ml of levetiracetam.

### VII. *Photo stability (Sun light and UV light)*

Levetiracetam tablet powder was exposed to sunlight and UV radiation to determine the effects of light irradiation on the stability of Levetiracetam in the solid state. Approximately 100 mg of Levetiracetam tablet powder was spread on a glass dish in a layer that was less than 2 mm thick. All samples for photo stability testing were placed in direct sunlight exposed for 12 hours and under UV cabinet (At $25^0$C with both UV radiation shorter and higher wavelength 210 and 310, respectively) for 12 hours. The tablet powder were removed from the sun and UV light and the contents of powder equivalent to 10 mg of Levetiracetam was accurately weighed and transferred to volumetric flask to make the final concentration 100 µg/ml of levetiracetam. Control samples which were protected with aluminium foil were also placed in the light cabinet and exposed concurrently. Following removal from the light cabinet, all samples were prepared for analysis as previously described [4].

## 3. Result and Discussion

### 3.1 *Determination of wavelength maxima*

Solution of levetiracetam was scanned between 200 and 400 nm with the help of PDA detector. UV spectra of the levetiracetam show maximum absorbance at 212 nm figure 2.

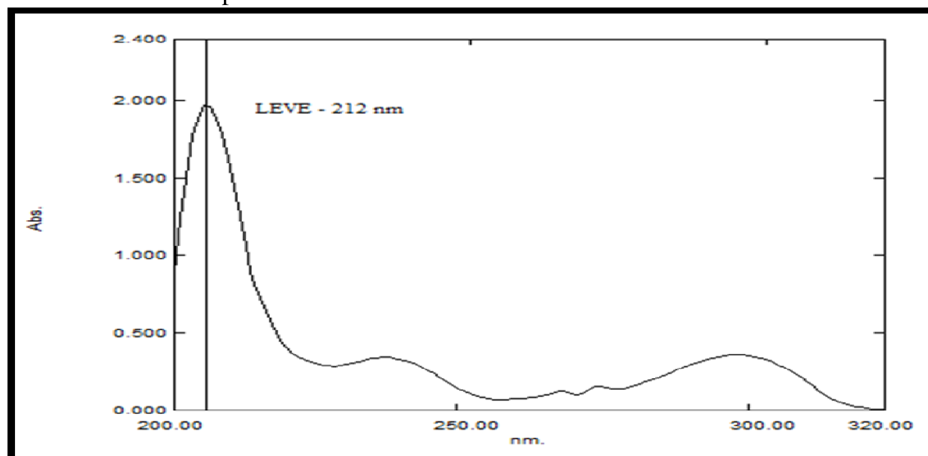


**Figure 2: UV spectra of Levetiracetam between 200 and 400 nm by UV-Visible spectrophotometer**

### 3.2 *Force degradation of levetiracetam*

Singh and Bakshi, on stress testing suggested a target degradation of 20-80 % for the establishing stability indicating nature of the assay method, as even intermediate degradation products should not interfere with any stage of drug analysis. Though conditions used for forced degradation were attenuated to achieve degradation in the range of 20-80 %, this could not be achieved in some cases even after exposure for prolonged duration. Levetiracetam showed extensive degradation in acidic, basic, wet and oxidative condition within very short time. Table 1. indicates the extent of degradation of levetiracetam under

various stress conditions. *Stability data for the degradation of levetiracetam were analyzed according to the ICH guidelines.*

| Stress condition/ state | Time | % Assay of active substance | Retention time (RT) | | | Relative retention time (RRT) | | |
|---|---|---|---|---|---|---|---|---|
| | | | IMP-A | IMP-B | LEVE | IMP-A | IMP-B | LEVE |
| **Acidic 0.1 N HCL (60°C)/solution** | 4 hr | 66.565 | 3.188 | 3.881 | 5.343 | 0.657 | 0.745 | **1.0** |
| **Alkali 0.1 N NaOH (60°C)/solution** | 4 hr | 68.837 | 3.198 | 3.884 | 5.302 | 0.659 | 0.746 | **1.0** |
| **Wet heat (60°C)/solution** | 4 hr | 62.484 | 3.182 | 3.878 | 5.342 | 0.632 | 0.726 | **1.0** |
| **Oxidative 3% $H_2O_2$ (60°C)/solution** | 8 hr | 62.024 | 3.194 | 3.888 | 5.334 | 0.645 | 0.736 | **1.0** |
| **Dry hear (60°C)/solid** | 24 hr | 61.716 | 3.132 | 3.834 | 5.378 | 0.665 | 0.753 | **1.0** |
| **Photo light/solid** | 12 hr | 66.343 | 3.185 | 3.879 | 5.320 | 0.643 | 0.734 | **1.0** |
| **UV Light 254 and 310 nm/solid** | **12 hr** | **69.956** | **3.184** | **3.864** | **5.302** | **0.641** | **0.732** | **1.0** |

**Table: 1. Force degradation summary of Levetiracetam by stability indicating RP-HPLC method**

### 3.3 *Degradation behaviour of levetiracetam*

In total, three degradation products were detected by RP-HPLC on decomposition of the drug under various stress conditions. Levetiracetam showed extensive degradation in acidic, basic, wet and oxidative condition. The degradation behaviour, retention time (RT) and relative retention time (RRT) of the drug and the degradation products are listed in Table: 1. The degradation behaviour of the drug in individual stress conditions is outlined below. HPLC studies of samples obtained on stress testing of levetiracetam under different conditions using methanol : water : acetonitrile (30:10:40) as the mobile solvent system suggested for the following degradation behaviour. Stability study in all the force degradation condition show formation of two common impurities at retention time of 3.188 and 3.885 for Impurity-A and Impurity-B, respectively as depicted in Figures 3-5 and table 2.

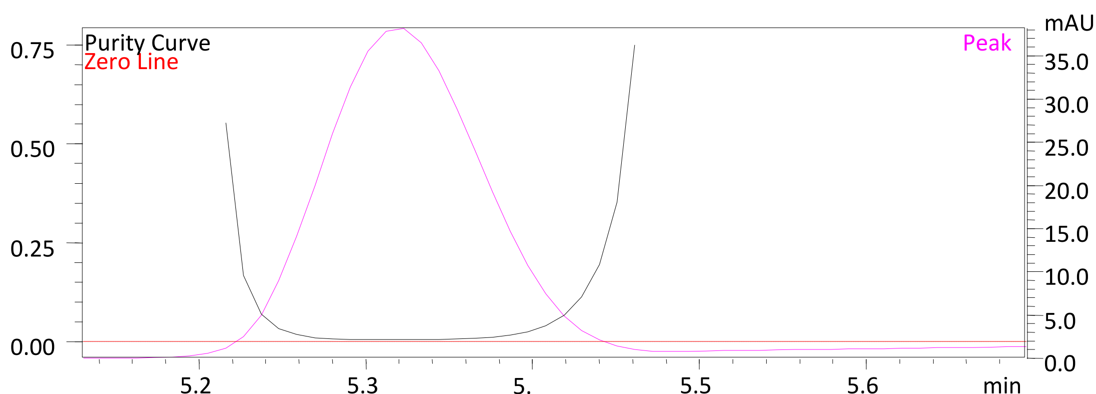


**Figure 3: Purity plot of Levetiracetam at 212 nm by stability indicating RP-HPLC method**

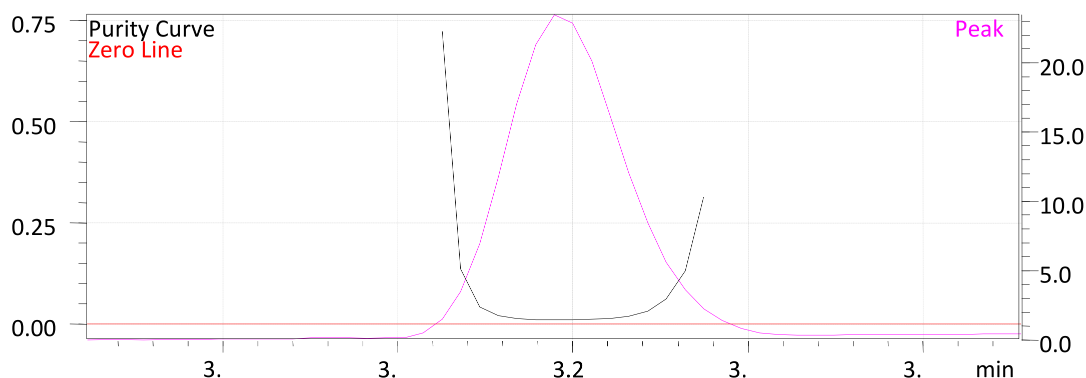


**Figure 4: Purity plot of impurity A at 212 nm by stability indicating RP-HPLC method**

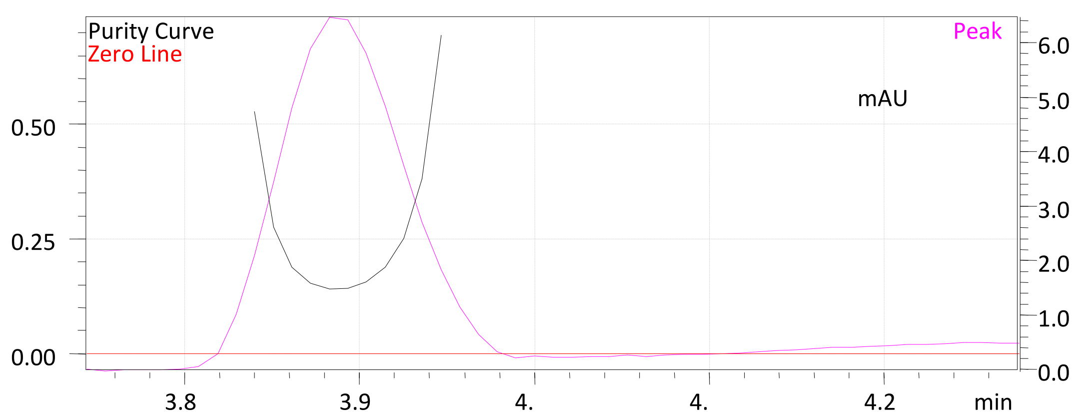


**Figure 5: Purity plot of impurity B at 212 nm by stability indicating RP-HPLC method**

**Table 2: Peak purity data of Levetiracetam by stability indicating RP-HPLC method**

| Drug/degradation Products | Peak purity index | Single point threshold | Minimum peak purity threshold |
|---|---|---|---|
| **Impurity-A** | 1.00 | 0.99081 | **9183** |
| **Impurity-B** | 1.00 | 0.97942 | **10478** |
| **Levetiracetam** | **0.99992** | **0.99478** | **5209** |

### 3.4 *Acid degradation*

It was observed that around 30–40 % of the drug degraded on refluxing it in methanolic 0.1N HCl for 4 hr and there was corresponding formation of degradation products (Figure 7) as compared to the chromatogram of drug in formulation (Figure 6). Two new peaks were seen in the chromatograms of acid-degraded samples of levetiracetam, as compared to the standard (Figure 6). This indicates that the drug degraded to low molecular weight compounds. The drug concentration gradually decreased with time on refluxing in 0.1N HCl and formation of degraded products increases as the time pass.

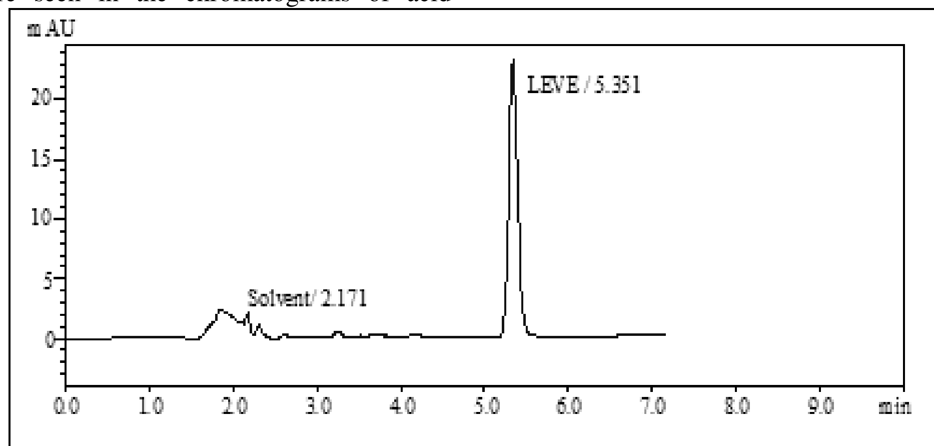


**Figure 6: Chromatogram of Levetiracetam standard (1µg/ml) with corresponding retention time at 212 nm by RP-HPLC method**

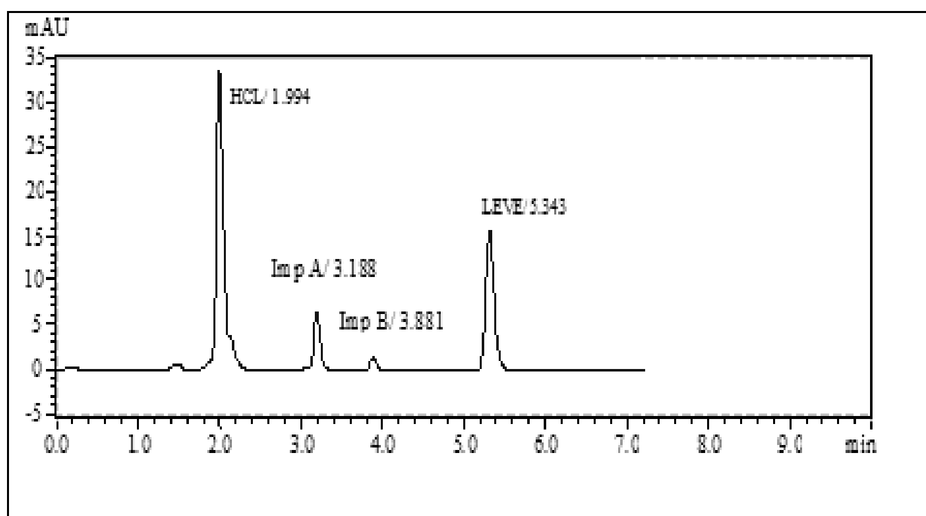


**Figure: 7 Chromatogram of acid degradation of Levetiracetam at 212 nm by stability indicating RP-HPLC method**

### 3.5 *Alkali degradation*

The results obtained on degradation of levetiracetam in alkaline conditions were found to be very similar to those reported for acidic condition. 30-40 % degradation was observed within 4 hr refluxing in methanolic 0.1N NaOH. Two new degraded impurities were separated from the drug. The product was seen during HPLC analyses of alkali-degraded samples with drug (Figure 8) and compared with (Figure 6), chromatogram of Standard drug. The drug was found to be highly labile to alkaline condition. The rate of degradation in 0.1N NaOH was as fast as acid condition. Drug degradation was associated with rise in a major degradation product.

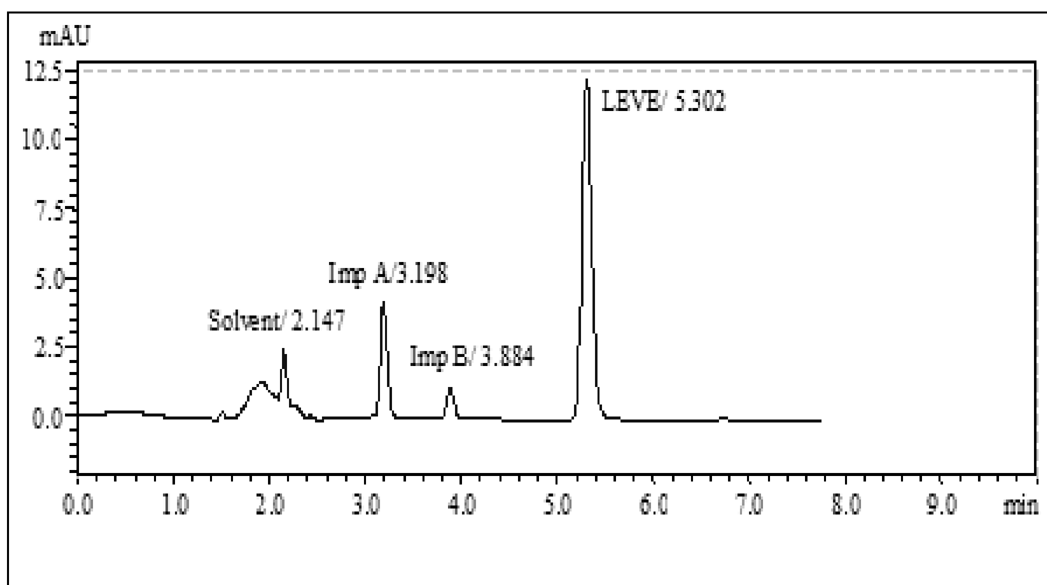


**Figure: 8 Chromatogram of alkali degradation of Levetiracetam at 212 nm by stability indicating RP-HPLC method**

### 3.6 *Wet heat (Hydrolysis)*

In neutral condition, only 20–25 % degradation of the drug was seen after refluxing at 60ºC for at the end of 4 h, degradation of the drug was observed with the corresponding rise in the major degradation peak (Figure 9). The degraded impurities observed in the wet degradation were not differing from the degradation of acid and alkali. The chromatogram was compared with standard and formulation.

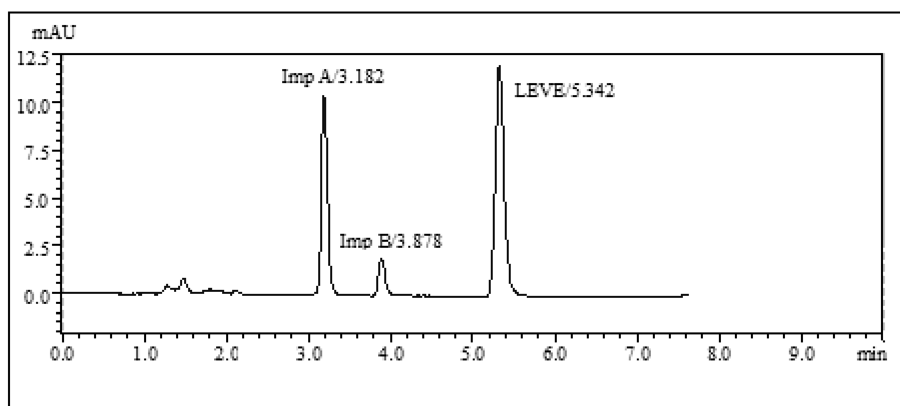


**Figure: 9 Chromatogram of wet degradation of Levetiracetam at 212nm by stability indicating RP-HPLC method**

### 3.7 *Oxidation*

Almost 25-35% drug degradation was observed on exposure to 3% $H_2O_2$ for 8 h. One major quantity degradation product peaks at around 3.194 minute was seen, and also there was significant rise in the height and area of these peaks with time (Figure 10). The major peak observed at retention time of 2.001 RT was compared with blank chromatogram of 3% $H_2O_2$ indicates that peak was of $H_2O_2$ not of impurity. This showed that the drug was degraded in oxidative conditions, resulting in products resolving again at same RT as acid, alkali and wet degradation.

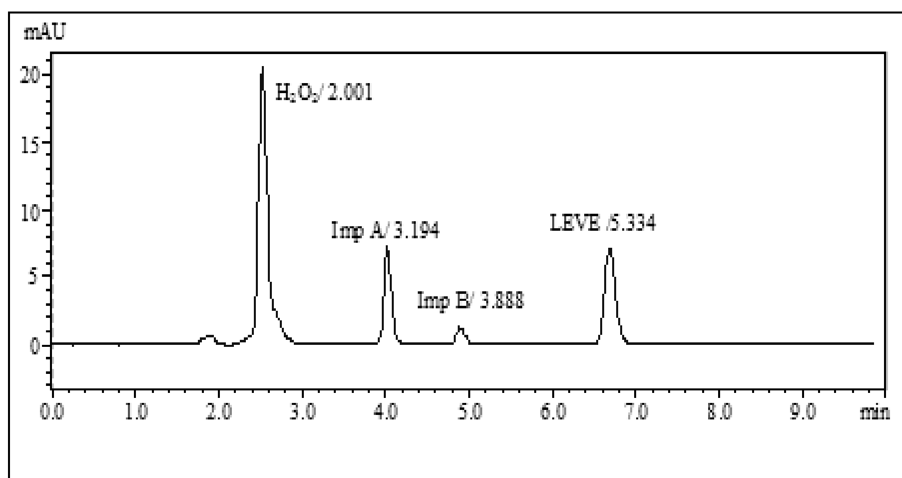


**Figure: 10 Chromatogram of oxidative degradation of Levetiracetam at 212 nm by stability indicating RP-HPLC method**

### 3.8 *Dry heat*

Levetiracetam was found to be stable as compare to acid, base and wet degradation after exposing the drug to 60ºC for 12 hours (Figure 11). The exposure of the solid drug to 60ºC for 24 hours shows around 40 % degradation. It indicated that levetiracetam was stable to dry heat than other force degradation condition.

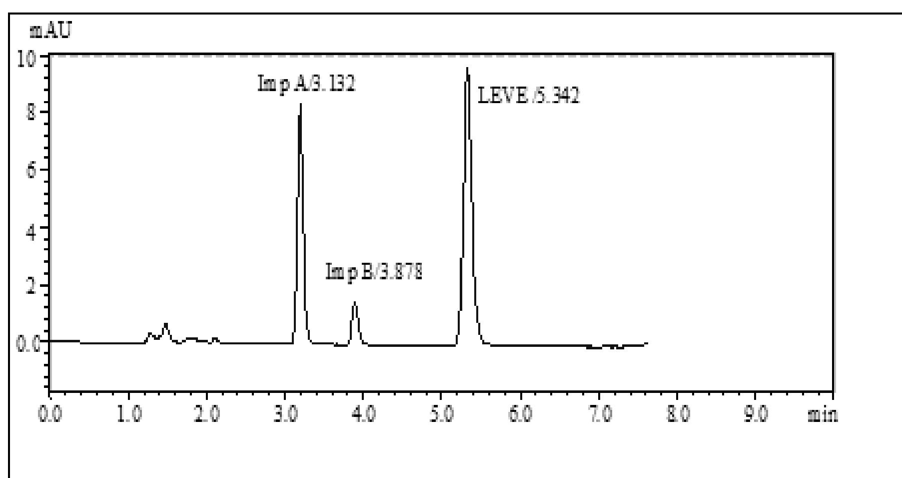


**Figure: 11 Chromatogram of dry heat 60 ºC degradation of Levetiracetam at 212nm by stability indicating RP-HPLC method**

### 3.9 *Photo stability (Photo and UV light)*

Levetiracetam was found to be labile on exposure to sunlight and UV light. In solid and methanolic condition 15-25 % degradation was observed. Under the conditions, the drug decomposed to the same major degradation product (Figure 12 & 13), both were also formed during hydrolysis, alkali and acidic, and dry heat degradation. No degradation was observed in control samples in the dark chamber. Two degradation product was observed after exposure of drug solution in sunlight and UV light for 12 hours, only minor degradation products observed instead of major one as in other force degradation were formed. Almost 20-30% of the drug degraded in 12 hours with formation of a two minor degradation products between retention time ranges of 3 to 4 minute. This peak was found to increase with time. In this case, the fall in drug peak was in correspondence with the rise in degradation product peaks.

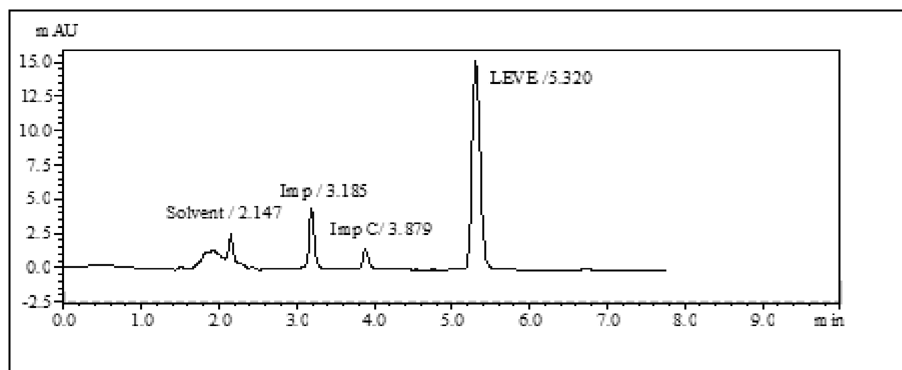


**Figure: 12 Chromatogram of sunlight degradation of Levetiracetam at 212nm by stability indicating RP-HPLC method**

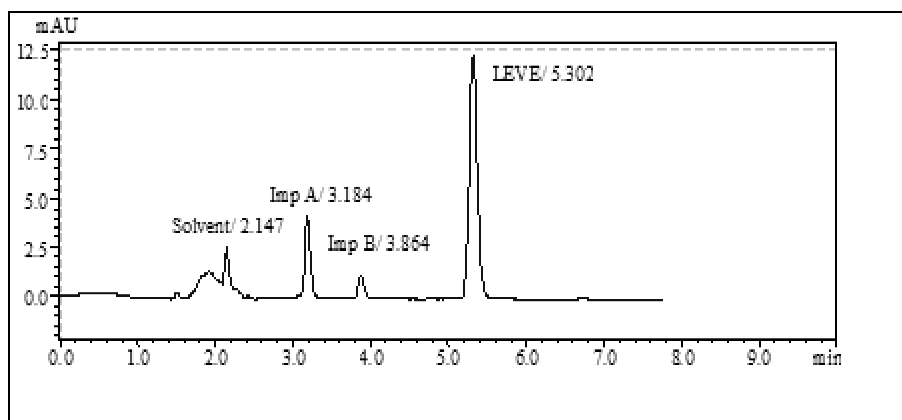


**Figure: 13 Chromatogram of UV light degradation of Levetiracetam at 212nm by stability indicating RP-HPLC method**

## 4.Conclusion

The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating. The proposed method is simple, accurate, precise and has ability to separate drug from degradation products and excipients found in the dosage forms.

## Conflict of interest statement

We declare that we have no conflict of interest.

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