

## Eco-friendly UPLC method for determination of Levetiracetam and its toxic related substance

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### ARTICLE INFORMATION



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### ABSTRACT

Nowadays, analytical chemistry society is aware of the hazardous influence of chemicals on both health and environment. Additionally, chemists are more interested in eco-friendly methods of analysis. Levetiracetam (LEV) is an antiepileptic drug with no significant interaction with other medications and so it is a safe treatment in elderly people. The toxic pyridine-2-ol is reported in British Pharmacopoeia to be LEV impurity C (IMP-C) and related substance. A highly sensitive eco-friendly UPLC method was introduced for the first time for analysis of LEV and its toxic IMP. Separation has been carried out on CN column using 0.1% aqueous sodium lauryl sulphate:acetonitrile (7:93, v:v) with UV scanning at 205 nm. USP recommendations for method validation have been followed with respect to linearity, accuracy, robustness and ruggedness. The developed method was successfully applied for quantitation of LEV in its tablets dosage form and statistical analysis with the reported method showed no significant difference at confidence limit of 95%. The short run time (<3 minutes) and high sensitivity are of the most advantageous of developed method over the reported one.

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### 1. Introduction

Levetiracetam ((S)-ethyl-2-oxo-1-pyrrolidine acetamide) (Figure 1) is used as an adjunctive treatment for drug-resistant partial seizures and it also may be used in other seizure types including myoclonic epilepsy [1,2]. LEV is a widely and safely used drug with no significant drug interaction [3]. In British Pharmacopoeia (BP) [1], pyridine-2-ol (Figure 1) is reported to be one of LEV impurities, IMP-C, and also is considered as LEV related substance.

Pyridine and its derivatives are stated to cause acute and chronic hazardous effects. Acute effects such as skin, eye, nose and throat irritation, while chronic effects include damaging of the developing fetus. In addition, it was reported that exposure to pyridine and its metabolites, hydroxy pyridines, may cause hepatotoxicity and nephrotoxicity [4].

Different analytical methods were described in the literature for the measurement of LEV either in pharmaceutical formulations or biological fluids. These methods include colorimetric [5], HPTLC [6], HPLC [7-12], LC-MS [13, 14], electrophoresis [3] and UPLC [15,16]. None of the cited methods has analyzed LEV in presence of its toxic impurity.

Recently, micellar chromatography is widely applied due to its low toxicity, high selectivity and its ability to separate simultaneously charged and uncharged solutes [17]. Sodium

lauryl sulphate is commonly used as a micellar media as it is highly soluble in water and can be easily removed from the chromatographic system [17].

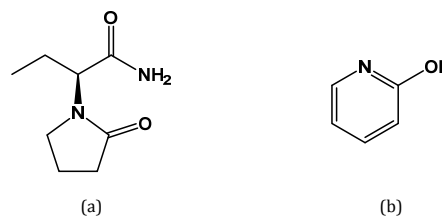


Figure 1. Chemical structure of (a) Levetiracetam and (b) its impurity

On the other hand, one of the main goals of green chemistry is to promote the use of "greener" solvents that are benign to environment and human health. Eco-friendly solvents are characterized by their low toxicity and high safety either to health or environment [18].

Due to the pharmaceutical importance of LEV and due to the toxic effects of its impurity, this work aims to develop and optimize a UPLC method for resolving the drug and its impurity with high sensitivity and selectivity. This is the first developed work for determination of LEV and its related toxic

substance; additionally it has the advantages of using green solvents and short analysis time.

## 2. Experimental

### 2.1. Instrument

Ultra performance liquid chromatographic separation was performed using a Thermo Scientific Dionex Ultimate S 3000 UPLC system with a stationary phase of Hypercil Gold Cyanide column with dimensions of 15 cm × 2.1 mm, and particle size of 3 µm (Germany).

### 2.2. Materials

#### 2.2.1. Pure samples

Levetiracetam was supplied from Sigma Pharmaceutical Industries, Quesna, Menoufia, Egypt and was certified to have a purity of 99.54%. While pyridine-2-ol (IMP-C) was purchased from Sigma-Aldrich Chemie GmbH, Germany with a purity of 99.90% according to the company certificates of analysis.

#### 2.2.2. Pharmaceutical formulation

Tiratam® tablets is labeled to contain 500 mg LEV and manufactured by Sigma Pharmaceutical Industries, Quesna, Menoufia, Egypt.

#### 2.2.3. Chemicals and reagents

All chemicals used were of analytical grade and solvents were of HPLC grade. Sodium lauryl sulphate (0.1 N aqueous solution) were purchased from LobaChemie Pvt. Ltd., India.

### 2.3. Solutions

*Stock standard solutions of LEV and IMP-C* (1 mg/mL): An accurately weighed amount of 0.1 g for each of LEV and its impurity were separately transferred in two 100 mL calibrated flasks, acetonitrile was added to dissolve the powders and the volume was adjusted with the same solvent.

*Working standard solution of LEV and IMP-C* (0.1 mg/mL): Working standard solutions were prepared by separately transfer 10 mL each from their corresponding stock standard solutions (1 mg/mL) into two 100 mL measuring flasks, the volume of each solution was adjusted with acetonitrile.

*Pharmaceutical formulation solution:* Ten Tiratam® tablets were weighed and finely grinded. An amount equivalent to 1 mg LEV has been weighed and then transferred to a 100 mL volumetric flask. The active ingredient was extracted from the excipients using acetonitrile. The solution was vortexed for 30 minutes, filtered through 0.45 µm membrane filter. The volume was then adjusted by the same solvent to prepare a stock solution of 1 mg/mL. Working solution was prepared in the concentration of 100 µg/mL LEV from which different dilutions within the linearity range were prepared.

### 2.4. Procedure

#### 2.4.1. Instrumental conditions

Separation was carried out on Hypercil Gold Cyanide column (15 cm × 2.1 mm, 3 µm particle size) at 25 °C using isocratic elution mode of a ecofriendly mobile phase composed of acetonitrile:0.1% sodium lauryl sulphate (97:3, v:v). The flow rate was pumped at 1 mL/min, UV scanning was carried out at 205 nm and 30 µL was the injection volume. The run time was set at 3 minutes.

#### 2.4.2. Construction of the calibration graph

Different samples of pure LEV and IMP-C in the concentration range of 1-50 µg/mL were separately prepared in the mobile phase and then injected in triplicates on the UPLC system under the specified instrumental conditions. Peak area was recorded, and then used to construct the calibration curves and calculating the regression equations.

#### 2.5. Method validation

After method optimization, the method has been validated regarding linearity, accuracy, precision, specificity, robustness and ruggedness following the instructions given by United States Pharmacopoeia (USP) guidelines [19].

#### 2.6. Application of the developed method

To test the validity of the developed method, it was applied for analysis of Tiratam® tablets. The previously prepared sample solutions were analyzed following the procedure under instrumental conditions. The recorded peak area of LEV and the previously computed regression equation have been used to calculate LEV content in Tiratam® tablets as % found. In addition, standard addition technique was carried out by addition of different concentrations of pure LEV on the pre-analyzed sample solution and the concentrations of the pure added LEV were then calculated as % found.

#### 2.7. System suitability

The performance of overall system was tested by calculating several parameters like asymmetry, selectivity, capacity and resolution factors.

#### 2.8. Statistical analysis

Statistical comparison between the developed method and the reported HPLC [7] one was performed using Student's *t*-test and *F*-test value.

## 3. Results and discussion

Determination of drugs in presence of their degradation products and impurities recently became an important point of interest for analysts especially if the drug degradations or impurities have hazardous effects [20-25]. Reviewing the published chromatographic methods for determination of LEV, acetonitrile was the organic modifier of choice and C18 column was the used stationary phase. Method optimization started with using C18 column (5 cm × 2.1 mm, 2 µm particle size) and an isocratic solvent mixture of acetonitrile:water, from 50% till 95% acetonitrile and a flow rate of 0.3 mL/min. In all trials, LEV and IMP-C were eluted with the mobile phase. Different pH values were then tried in the range of (3.5-8.0) but no improvement was observed. Changing the mobile phase flow rate had no effect. The stationary phase was then changed to CN-column (15 cm × 2.1 mm, 3 µm particle size). On using 85% aqueous acetonitrile pumping at a flow rate of 1 mL/min, LEV was eluted after 2.2 min while IMP-C after 3 min but with tailed peaks. After successive trials it was observed that increasing acetonitrile improved the chromatographic resolution with slight improvement in peak shape while water significantly improved the shape of the separated peaks however it resulted in bad resolution and early elution of LEV. Different concentrations of sodium lauryl sulphate (SDS) were then used to improve the peak asymmetry where 0.1% aqueous SDS was the concentration of choice. The optimum mobile phase was that consisted of 0.1% aqueous SDS:acetonitrile (7:93, v:v).

**Table 1.** Assay and method validation parameters for the determination of pure samples of levetiracetam and its impurity by the proposed method.

Parameters	Levetiracetam	IMP
Range ( $\mu\text{g/mL}$ )	1-50	1-50
Slope	0.6783	0.4372
Intercept	0.1264	0.0113
Correlation coefficient ( $r$ )	0.9998	0.9997
Accuracy (% Recover) <sup>a</sup>	101.44	101.63
Precision (SD)		
Repeatability <sup>b</sup>	0.520	2.125
Intermediate precision <sup>c</sup>	1.803	2.408
LOD ( $\mu\text{g/mL}$ ) <sup>d</sup>	0.30	0.32
LOQ ( $\mu\text{g/mL}$ ) <sup>d</sup>	0.90	0.96

<sup>a</sup> Accuracy was expressed as mean percentage recovery and it was performed on nine different samples (in triplicates) each of pure Levetiracetam and IMP-C.

<sup>b</sup> Standard deviation of 3 concentrations of each drug (5, 10 and 25  $\mu\text{g/mL}$ ) analyzed in triplicates on the same day,  $n = 9$ .

<sup>c</sup> Standard deviation of 3 concentrations of each drug (5, 10 and 25  $\mu\text{g/mL}$ ) analyzed in triplicates on three successive days,  $n = 9$ .

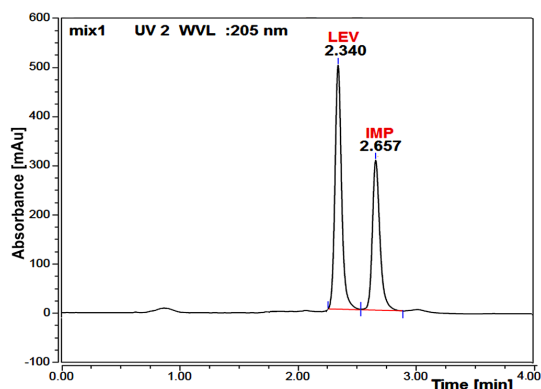
<sup>d</sup> LOD =  $3.3 \times \text{S.D./Slope}$  while LOQ =  $10 \times \text{S.D./Slope}$ , they were calculated using the lower part of the calibration graphs.

**Table 2.** Determination of levetiracetam in its dosage form by the proposed method and application of standard addition technique.

Pharmaceutical formulation	Taken ( $\mu\text{g/mL}$ )	Found <sup>a</sup> % $\pm$ S.D.	Added ( $\mu\text{g/mL}$ )	Found (%) <sup>b</sup>
Tiratam <sup>®</sup> tablets labeled to contain 500 mg LEV/tablet	20.00	101.26 $\pm$ 0.900	10.00	98.14
			15.00	100.93
			20.00	103.12
			25.00	98.55
	Mean $\pm$ S.D.			100.19 $\pm$ 2.311

<sup>a</sup> Average of 5 determinations.

<sup>b</sup> Average of 3 determinations.

**Figure 2.** UPLC Chromatogram of Levetiracetam and its impurity.

From the chemical structure of LEV (Figure 1), it was observed that its structure contains no chromophoric group and hence detection wavelength played a significant role in method sensitivity. Different scanning wavelengths (200, 205, 210 and 220 nm) were tried. Maximum sensitivity with minimum noise was obtained on detection at 205 nm.

Finally, separation was performed on CN-column using a mobile phase of 0.1% aqueous SDS: acetonitrile (7:93, v:v), flow rate of 1 mL/min and detection at 205 nm. LEV appeared at  $2.34 \pm 0.004$  min while IMP at  $2.657 \pm 0.003$  with acceptable shape and resolution, Figure 2.

After method optimization, linearity was tested where good correlation coefficients were obtained between the recorded peak area versus the concentration in the range of 1-50  $\mu\text{g/mL}$  for both LEV and IMP, Figure 3 and the following regression equations have been calculated:

$$A = 0.6783 \times C_{\text{LEV}} + 0.1264 \quad (r = 0.9998) \quad (1)$$

$$A = 0.4372 \times C_{\text{IMP}} + 0.0113 \quad (r = 0.9997) \quad (2)$$

where A is the area under the peak, C is the concentration in  $\mu\text{g/mL}$  and  $r$  is the correlation coefficient.

Validation of the method was carried out where good accuracy was observed as 101.44 and 101.63% for LEV and IMP-C, respectively, Table 1.

The values of the calculated SD for repeatability and intermediate precision were  $< 2.5$ , Table 1 indicating that the developed method was precise. Specificity was also confirmed by the good resolution obtained among the two separated peaks. Moreover, no interference from excipients was observed on applying the method to pharmaceutical dosage form, Table 2.

On testing method sensitivity, detection and quantitation limits were calculated where low values were obtained. Table 1 was confirming the high sensitivity of the developed chromatographic method. Robustness and ruggedness were also tested where the obtained results were presented in Table 3, all the obtained SD values were within the acceptable limit (%RSD  $< 4\%$  [26]) indicating that the studied parameters or conditions have no significant effect on peak area of both LEV and IMP-C.

When the optimized chromatographic method was applied to Tiratam<sup>®</sup> tablets, the resulted concentrations were found to be within the acceptable limits ( $101.26 \pm 0.900$ ), Table 2 proving that tablets additives did not interfere. Standard addition technique was used to further confirm method accuracy where good results were obtained, Table 2 and verified good accuracy of the developed method.

System suitability testing parameters were calculated in order to confirm that the overall system is performing well.

**Table 3.** Robustness and ruggedness studies of the developed method.

Factor	Levetiracetam	IMP
Robustness (S.D.)*		
Mobile phase flow rate ( $\pm 0.05$ min)	3.213	3.188
% aqueous SDS in the mobile phase ( $\pm 0.5\%$ )	1.340	2.450
Ruggedness (S.D.)*		
Two analysts	1.272	2.341

\* SD of peak area.

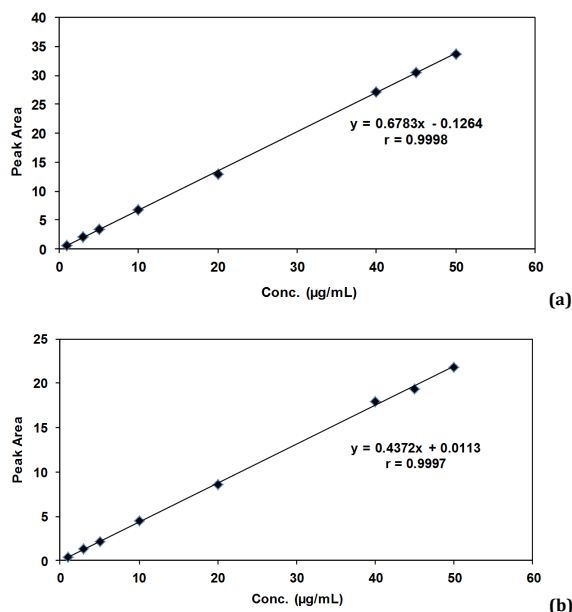
**Table 4.** System suitability testing parameters of the developed method.

Parameters	Levetiracetam	IMP
$t_R$ (min)	2.377 $\pm$ 0.004	2.703 $\pm$ 0.003
Peak symmetry	1.40	1.32
Capacity factor (K')	1.98	2.38
Selectivity ( $\alpha$ )	1.20	1.20
Resolution ( $R_s$ )	3.54	3.54
Number of theoretical plates (N)	15537	12405
Height equivalent to theoretical plate (h) (in cm)	0.00097	0.00121

**Table 5.** Statistical comparison of the developed method and the reported one.

Parameters	Levetiracetam	Reported method [7]
Mean	101.44	101.02
n	9	9
SD	1.227	1.258
Student's <i>t</i> -test (2.120)	0.717	-
F-value (3.438)	1.051	-

Reported method: HPLC analysis using a mobile phase consisted of potassium dihydrogen phosphate buffer (50 mM, pH = 4.5): acetonitrile (94:6, v:v), stationary phase of Hydro-RP, 150 mm  $\times$  4 mm I.D. using a flow rate of 1.5 mL/min and detection wavelength of 205 nm.

**Figure 3.** The calibration graphs of the recorded peak area versus the concentration in the range of 1-50  $\mu\text{g/mL}$  for both LEV (a) and IMP-C (b).

The value of the calculated parameters were within the acceptable limits where the calculated peak symmetry for both LEV and IMP-C  $< 1.5$ , capacity factors (between 1-10), selectivity factors  $>1$  and resolution between the two eluted peaks was  $>2$ , Table 4.

The method compared favorably with the reported RP-HPLC method [7] when statistical analysis was carried out and it was found that the obtained values of student's *t*- and F-test were less than the tabulated ones confirming that there was no significant difference between the two methods, Table 5.

The proposed method was the first developed one for separating the drug and its harmful impurity. Unlike other reported methods it has advantageous of short analysis time, simple sample pretreatment steps. Also, it has higher sensitivity comparing to the reported HPLC one [7]. In addition, the used mobile phase is simple, easily prepared and

did not need pH adjustment comparing to the published UPLC methods [15,16].

#### 4. Conclusion

This work represents a newly developed eco-friendly UPLC method for the separation and quantitation of LEV and its toxic impurity. From the advantages of the developed method are its short analysis time with high sensitivity, accuracy and precision. The suggested method has been successfully applied for the measuring of LEV in pure form and in its tablet dosage form. The used mobile phase is eco-friendly and easily to be prepared, hence the method is suitable for application in quality control laboratories during drug analysis.

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