
Research Article



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Spectrophotometric Determination of Levetiracetam Using 2, 4-DNP and MBTH in Pharmaceutical Dosage Form**R.S. Chandan*, M. Indupriya, P.Lavanya***Department of Pharmaceutical Analysis, JSS Academy of Higher Education & Research, JSS College of Pharmacy, Mysore 570 015, (KA), India.*

ABSTRACT**Purpose**

This study was aimed to evaluate the method of Spectrophotometric Determination of Levetiracetam Using 2, 4-DNP and MBTH in Pharmaceutical Dosage Form.

Findings

Levetiracetam at its λ_{max} 455 nm shows linearity in the concentration range of 30-130 $\mu\text{g mL}^{-1}$. Second method is based on reaction of oxidative coupling of levetiracetam with 3-Methyl-2-Benzthiazolinone hydrochloride (MBTH) to form green colored product. Levetiracetam at its λ_{max} 634 nm shows linearity in the concentration range of 20-100 $\mu\text{g mL}^{-1}$. The relative standard deviations for first method is 0.422% and for second method is 0.325% were obtained.

Conclusion

The reliability and performance of the proposed methods was validated statistically the percentage recovery ranged from 99.88 and 100.2 respectively. The results of analysis for the two methods have been validated statistically and by recovery studies.

Keywords: Spectrophotometric, Oxidative coupling, 2, 4-Dinitrophenyl hydrazine (2, 4-DNP), 3-Methyl-2-Benzthiazolinone hydrochloride (MBTH), Levetiracetam. Reliability, Statistically

INTRODUCTION

Levetiracetam [1](LEV) is a novel antiepileptic agent; with a chemical name (S)-(2)-(2-oxopyrrolidin-yl) butamide (figure 1). It is used as an adjunctive therapy in the treatment of partial

seizures [2]. Levetiracetam can prevent myoclonic jerks and generalized epileptiform activity in patients with photosensitive epilepsy. The precise mechanism by which levetiracetam exerts its antiepileptic effect is unknown. However the drug binds to a synaptic vesicle protein, (SV2A) [3],

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which is believed to impede nerve conduction across synapses [4].

The therapeutic importance of levetiracetam was behind the development of numerous methods for its determination. Literature survey reveals that various HPLC [5-10] and LC-MS [11-13] methods have been reported for the determination of levetiracetam in pure and pharmaceutical dosage forms. These methods require long and tedious pre-treatment of the samples and laborious clean up procedures prior to analysis. An official monograph of LEV does not exist in any pharmacopoeia and determination of LEV in bulk and pharmaceutical formulations has not been yet described. A through literature search has revealed that only few spectrophotometric methods available for determination of levetiracetam in bulk drugs and pharmaceutical formulations. So there is a need for development of simple and suitable analytical spectrophotometric method for the determination of LEV in bulk and pharmaceutical formulations. UV-Visible spectrophotometry is the technique of choice in research laboratories, hospitals and pharmaceutical industries due to its low cost and inherent simplicity.

2, 4-Dinitrophenyl hydrazine (2, 4-DNP) [14] and 3-Methyl-2-Benzthiazolinone hydrochloride (MBTH) [15-17] and have been used as chromogenic reagents for the spectrophotometric determination of pharmaceutical dosage forms. However, the reactions of 2, 4-DNP and MBTH with LEV have not been investigated so far. The present study describes the evaluation of 2, 4 DNP and MBTH as chromogenic reagents in the development of simple and rapid spectrophotometric method for the determination LEV in its pharmaceutical dosage forms.

MATERIALS & METHODOLOGY

Apparatus

A Shimadzu UV-visible spectrophotometer model 1800 with 1 cm matched quartz cell was used for the absorbance measurements. Shimadzu electronic balance was used for weighing the samples.

Reagents and solutions

All employed chemicals were of analytical grade and high-purified water was used throughout. Levetiracetam pure sample was obtained as a gift

sample from Intas Pharmaceuticals Ltd., Gujarat, India.

Standard solutions

In both methods levetiracetam stock solution ($1000 \mu\text{g mL}^{-1}$) was prepared by dissolving 100mg of drug in 100mL of methanol. Working solutions of the drug were prepared by dilution of the stock solution. The marketed tablet form of LEV used in the determination was TORLEVA 250 with a labelled strength of 250 mg and manufactured by Torrent Pharmaceuticals Limited, Dist. Solan (H.P), India.

Reagents 2, 4-Dinitrophenyl hydrazine (2, 4-DNP) 0.08 % (w/v)

A 0.08% w/v of the reagent solution were freshly prepared by dissolving 0.08 g of 2, 4 DNP in 2 mL of concentrated H_2SO_4 and diluting to 100 mL with water.

10N Sodium hydroxide solution

40 g of sodium hydroxide dissolve in 100 mL of distilled water.

Potassium iodate 4 % (w/v)

A 4% w/v potassium iodate solution was prepared by dissolving 4 g in 100 mL of distilled water.

3-Methyl-2-Benzthiazolinone hydrochloride (MBTH) 0.5 % (w/v)

0.5 g of MBTH reagent was accurately weighed transferred into a 100 mL calibrated flask, dissolved in distilled water, and make up the volume up to the mark to obtain a solution of 0.5% (w/v).

Ferric chloride (1%)

Freshly prepared was prepared by dissolving 1 g of ferric chloride in 100 mL of distilled water.

General recommended procedures

Procedure for calibration graph

Method 1

Standard solutions of LEV in methanol, having final concentrations in the range of 30-130 $\mu\text{g mL}^{-1}$ were transferred into a series of 10 mL volumetric flasks, to these solutions 1.5 mL of 2,4-DNP(0.08%) and 1.5 mL of KIO_3 (4%)were

added, which were made alkaline by adding 1 mL each of NaOH (10N). The red color hence developed was further diluted to the volume with water. The absorbance of each solution was measured at 455 nm against the reagent blank prepared in the same manner, without the analyte and the calibration curve and absorption spectra are represented in Figure. 2 and Figure. 3.

Method 2

Standard solutions of LEV in methanol, having final concentrations in the range of 20-100 $\mu\text{g mL}^{-1}$ were transferred into a series of 10 mL volumetric flasks. To each 2 mL of MBTH, 2 mL of ferric chloride was added and the volume was made up to mark with distilled water and allowed to stand for 20 minutes. The contents were diluted up to 10 mL with water. The absorbance of each solution was measured at 634 nm against the reagent blank. The colored species was stable for 2 h and the amount of drug in the sample was computed from its calibration curve and absorption spectra represented in Figure. 4 and Figure. 5.

Procedure for pharmaceutical formulations

Method 1

Twenty tablets were weighed and their contents are mixed thoroughly. An accurately weighed portion of powder equivalent to the 100 mg of LEV was weighed into a 100 mL volumetric flask containing about 50 mL of methanol. It was shaken thoroughly for about 5-10 minutes, filter thoroughly with Whatman filter paper to remove insoluble matter and diluted to the mark with methanol to prepare 1000 $\mu\text{g mL}^{-1}$ solution. An aliquot of this solution was diluted with water to obtain a concentration of 60 $\mu\text{g mL}^{-1}$. Then to that solution 1.5 mL of 0.08 % 2, 4-DNP is added, and 1.5 mL of 4 % KIO_3 and 1 mL of 10N NaOH was added. The mixture was then gently shaken until the appearance of red color. The contents were diluted up to 10 mL with distilled water.

Method 2

Twenty tablets were weighed and their contents are mixed thoroughly. An accurately weighed portion of powder equivalent to the 100 mg of LEV was weighed into a 100 mL volumetric flask containing about 50 mL of methanol. It was shaken thoroughly for about 5-10 minutes, filter thoroughly with Whatman filter paper to remove

insoluble matter and diluted to the mark with methanol to prepare 1000 $\mu\text{g mL}^{-1}$ solution. An aliquot of this solution was diluted with water to obtain a concentration of 40 $\mu\text{g mL}^{-1}$. Then to that solution 2 mL of 0.5% MBTH, 2 mL of 1% FeCl_3 is added. The mixture was then gently shaken and the appearance of green color occurs. The contents were diluted up to 10 mL with distilled water. The results for determination of Levetiracetam in formulations and statistical comparison with the reference are given in table 5.

DISCUSSION

The absorption spectra of the reaction product of oxidized 2, 4 DNP with drug show maximum absorption (λ_{max}) at 455 nm. The blank solution was slightly red in color that had negligible absorbance at the λ_{max} in which the drug was analysed. Thus formed color was stable for more than two hours. A temperature range of 20-30 $^{\circ}\text{C}$ is preferred for the reaction. The 2, 4 DNP is oxidized by KIO_3 to give diazonium cation which reacts with drug by electrophilic substitution to give deep colored chromogen. Beer's law is obeyed in the range of 30-130 $\mu\text{g mL}^{-1}$ for LEV. In second method, the drug reacts with MBTH in the presence of FeCl_3 to give a green colored product. Actually, this is an iron catalyzed oxidative coupling reaction of MBTH with the drug. Under the reaction conditions, on oxidation, MBTH loses two electrons and one proton forming an electrophilic intermediate, which is the active coupling species. This intermediate undergoes electrophilic substitution with the drug to form the colored product. The colored products were found to be stable for 18 hours, at room temperature. Reproducible results were obtained in the temperature range of 20-40 $^{\circ}\text{C}$. The reagent blank has negligible absorbance in the range used for detection of the LEV. Beer's law is obeyed in the range of 30-130 $\mu\text{g mL}^{-1}$ for LEV.

In the present investigation, 2, 4-DNP and MBTH reagent forms colored complexes with LEV and their absorbances were measured at 455 nm and 634 nm respectively. Because of the presence of keto group as chromophoric group in the LEV molecule, oxidation of the compound was attempted with 2, 4-DNP as a result colored complex has been formed which was estimated

spectrophotometrically. And an oxidative coupling reaction takes place with LEV with MBTH reagent. Therefore, the present study was devoted to explore 2, 4-DNP and MBTH reagent as oxidative coupling reagents for the determination of LEV in pure and pharmaceutical dosage forms.

Optimization of the spectrophotometric conditions was intended to take into account the various goals of method development. Analytical conditions were optimized via a number of preliminary experiments.

For method A

By varying one and keeping other experimental parameters and the amount of drug constant, the effect of 2, 4-DNP, oxidizing agents and NaOH were studied. Maximum color intensity was obtained when 1.3-1.5 mL of 2, 4 DNP and 1.5-1.7 mL of potassium iodate were added to LEV. Different concentrations of sodium hydroxide were used for maximum color development and were found that 1 mL of 10 N NaOH was optimum.

For method B

The optimum conditions for the reaction were carefully studied. Maximum absorption at 634 nm was obtained immediately upon using 2 mL of 1% FeCl₃ and 2 mL of 0.5% MBTH at ambient temperature and the product remained stable for up to 35 minutes.

Optimization of parameter

The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance. Different concentrations and different volumes were tried for all the reagents, by varying the parameters at a time. For method 1 it was found that optimum concentration of KIO₃ was 4% w/v and optimum concentration of 2, 4-DNP was 0.08% w/v and optimum concentration of NaOH was 10N. The optimum volume was found to be 1.5 mL for KIO₃ and that of 2, 4-DNP was 1.5 mL and NaOH was 1 mL. For method 2 it was found that optimum concentration of MBTH reagent was 0.5% w/v and optimum concentration of FeCl₃ was 1% w/v. The optimum volume was found to be 2 mL for MBTH and that of FeCl₃ was 2 mL.

Stability of the Chromogen

Method 1

Under the optimum conditions, the reaction between LEV and 2, 4-DNP was completed within 2 minutes at room temperature, and the absorbance no longer changed after standing for up to 40 minutes. The effect of time on the stability of the chromogen was studied by following the absorption intensity of the reaction solution (after dilution) at different time intervals. It was found that the absorbance of the chromogen remains stable for at least 2 hours. This allowed the processing of large batches of samples and their comfortable measurements with convenience. This increased the convenience of the methods as well as made it applicable for large number of sample.

Method 2

The reaction between LEV and MBTH completed within 20 minutes. The green colour developed was found to be stable for long period and showed no change in the colour intensity with time. This allowed the method to be followed for the intra-day studies.

Quantification

The limits of the Beer's law, the molar absorptivity and the Sandell's sensitivity values were evaluated and are given in Table 1. Regression analyses of the Beer's law plots at their respective λ_{max} values revealed a good correlation. Graphs of absorbance versus concentration showed zero intercept, and are described by the regression equation, $Y = bX + c$ (where Y is the absorbance of a 1 cm layer, b is the slope, c is the intercept and X is the concentration of the drug in $\mu\text{g mL}^{-1}$) obtained by the least-squares method. The results are summarized in Table 1.

Validation of the method

The validity of the methods for the assay of LEV examined by determining the precision and accuracy. These were determined by analyzing six replicates of the drug within the Beer's law limits. The low values of the relative standard deviation (R.S.D.) indicate good precision of the methods. The results are given in Table 2 and 3. The average percent recoveries obtained were quantitative indicating good accuracy of the methods.

Linearity

To establish linearity of the proposed methods, a series of solutions of levetiracetam for method 1 ($30\text{--}130\ \mu\text{g mL}^{-1}$), method 2 ($20\text{--}100\ \mu\text{g mL}^{-1}$) and were prepared from the stock solutions and analyzed. Least square regression analysis was performed on the obtained data.

Precision

The precision of the proposed methods was ascertained by actual determination of six replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method.

Accuracy

The accuracy of the method is the closeness of the measured value to the true value for the sample. To determine the accuracy of the proposed method, different levels of drug concentrations three serial dilutions were prepared from independent stock

solutions and analyzed. Accuracy was assessed as the percentage relative error and mean % recovery. The accuracy and precision values are given in Table 4.

Ruggedness

To ascertain the ruggedness of the methods, four replicate determinations at different concentration levels of the drugs were carried out. The within-day RSD values were less than 1% and this indicate that the proposed method has reasonable ruggedness.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for levetiracetam by the proposed method were determined using calibration standards. LOD and LOQ were calculated as $3.3\ \sigma/S$ and $10\ \sigma/S$, respectively, Where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation. The results of LOD and LOQ are given in Table 1.

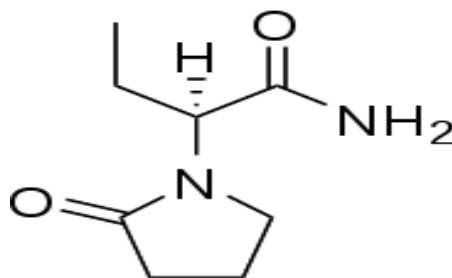


Figure 1: Structure of Levetiracetam

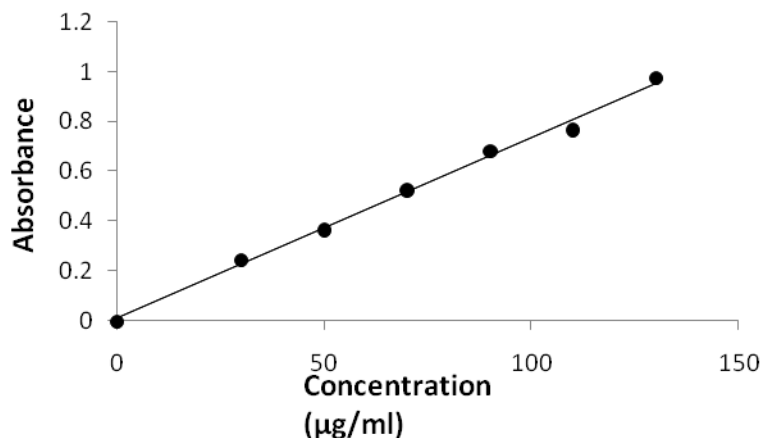


Figure 2: Calibration graph of levetiracetam (LEV) Conc. (LEV) = $30\text{--}130\ \mu\text{g mL}^{-1}$

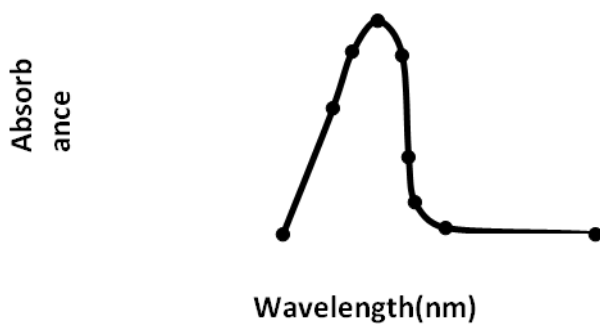


Figure 3: Absorption spectra of 2, 4-DNP with LEV against the reagent blank

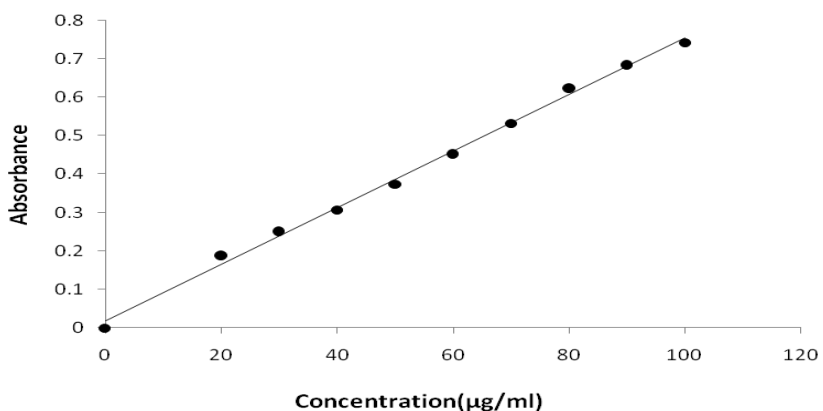


Figure 4: Calibration graph of Levetiracetam (LEV) Conc =20-100 µg mL⁻¹

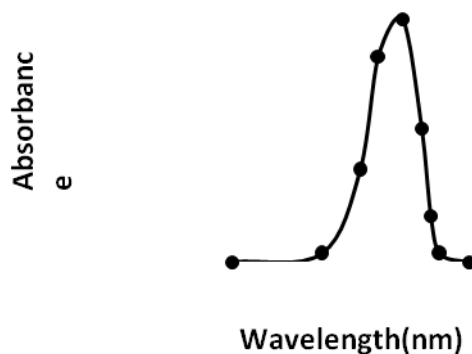


Figure 5: Absorption spectra of MBTH with LEV against the reagent blank

Table 1: Optical characteristics

S.NO	Parameter	Values	
		Method 1	Method 2
1	λ_{\max} / nm	455 nm	634 nm
2	Beers law limits ($\mu\text{g mL}^{-1}$)	30-130	20-100
3	Molar absorptivity (1 /mol/cm)	1.38996×10^{-3}	1.6085×10^{-3}
4	Correlation coefficient (R)	0.995	0.996
5	Sandell's sensitivity(ng cm^{-2})	0.0546	0.0892
6	Regression equation (y)	$Y=0.007x+0.010$	$y = 0.007x+0.018$
7	Slope, <i>b</i>	0.007	0.007
8	Intercept, <i>c</i>	0.010	0.018
9	Relative standard deviation%	0.422	0.325
10	% Range of error (95% confidence limits)	0.34	0.35
11	Limit of detection ($\mu\text{g mL}^{-1}$)	0.88	0.69
12	Limit of quantification($\mu\text{g mL}^{-1}$)	2.67	2.079

$Y = bX + c$, where *X* is the concentration of drug in $\mu\text{g mL}^{-1}$; Average of six determinations

Results of recovery study by standard addition method

Table 2: Recovery studies for LEV (method 1)

S.NO	Standard levetiracetam (mL)	Standard levetiracetam (μg)	Sample levetiracetam (mL)	Sample levetiracetam (μg)	Absorbance at 455nm	Amount of LEV from std. graph	Recovery of std (mg)	%Recovery
1	0.3	30	0.3	30	0.443	59	29	96.66%
2	0.4	40	0.3	30	0.615	82	42	105%
3	0.5	50	0.3	30	0.742	99	49	98%

Table 3: Recovery studies for LEV (method 2)

S.NO	Standard levetiracetam (mL)	Standard levetiracetam (μg)	Sample levetiracetam (mL)	Sample levetiracetam (μg)	Absorbance at 403nm	Amount of LEV from std. graph	Recovery of std (mg)	%Recovery
1	0.2	20	0.2	20	0.309	40.12	20.12	100.6%
2	0.3	30	0.2	20	0.448	59.7	29.7	99%
3	0.4	40	0.2	20	0.607	80.5	40.5	101%

Table 4: Evaluation of accuracy and precision-(Method 1)

S.no	LabelClaim (mg)	Amount found*	% Purity*	Average (%)	S.D	R.S.D ^a	R.S.D ^b	S.E.M
1	250	249.642	99.85	99.27	0.0018	0.422	0.653	0.17
2		245.208	98.08					
3		251.854	100.74					
4		244.689	97.87					
5		248.264	99.3					
6		249.548	99.81					

Table 5: Evaluation of accuracy and precision-(Method 2)

S.no	LabelClaim (mg)	Amount found*	%Purity*	Average (%)	S.D	R.S.D ^a	R.S.D ^b	S.E.M
1	250	248.231	99.29	99.81	0.0014	0.325	0.587	0.13
2		255.564	102.22					
3		246.423	98.56					
4		249.679	99.87					
5		247.554	99.02					
6		249.881	99.95					

SD. Standard deviation; SEM. Standard error of mean; RSD.relative standard deviation;

- Intraday precision,
- Interday precision.

Table 5: Results of determination of Levetiracetam in formulations and statistical comparison with the reference

Preparation	Label claim (mg per tablet)	Method	Percentage found	t-value	f-value
TORLEVA 250	250	Method A (2,DNP)	99.27	0.289	6.76
		Method B(MBTH)	99.81	0.419	4.0

CONCLUSION

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control was well

established by the assay of LEV in pure form and in pharmaceutical preparations.

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