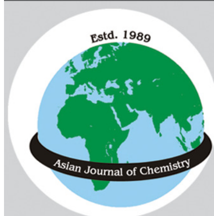
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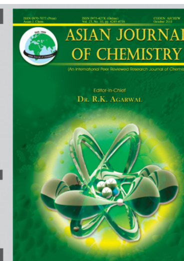

Spectrophotometric Determination of Levetiracetam by Developing Coloured Complexes with 2-Chlorophenylhydrazine and Anthranilic Acid

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Two simple, sensitive, reproducible, rapid and economical spectrophotometric methods are described for the determination of levetiracetam (LVC) in bulk and formulations. Both the methods are based on the formation of coloured complexes of levetiracetam with 2-chlorophenylhydrazine (**method-A**) and anthranilic acid (**method-B**) in alcoholic medium. Under the optimized conditions the complexes show an absorption maximum at 560 and 485 nm with molar absorptivities of 1.130×10^4 and 7.110×10^4 mol^{-1} cm^{-1} and sandells sensitivities of 0.03546 and 0.03708 per 0.001 absorbance unit for **method-A** and **method-B**, respectively. The result of analysis has been validated statistically and by recovery studies. Both methods have been successfully applied for the assay of the drug in pharmaceutical formulations.

Key Words: Levetiracetam, 2-Chlorophenylhydrazine, Anthranilicacid, Spectrophotometry and condensation.

INTRODUCTION

Levetiracetam (LVC) is chemically known as (S)-α-ethyl-2-oxo-pyrrolidine acetamide. It is a new antiepileptic drug (AED)[1], approved by the US food and drug administration. levetiracetam is the (s)-enantiomer and R-α-ethyl-2-oxo-pyrrolidine acetamide (REV) but only the (s)-enantiomer has anticonvulsant activity and consequently, only this enantiomer has been developed and used as a new antiepileptic drug[2]. It is an ethyl analogue of piracetam, a nootropic drug used as a cognition enhancer in the elderly and in the treatment of myoclonus[3]. In humans levetiracetam has proven efficacy in clinical trials of patients with partial and generalized seizures[4]. Levetiracetam has been regarded as a new antiepileptic drug with ideal pharmacokinetics[5]. In humans levetiracetam has a very low potential for drug-drug interactions[5] and it is eliminated mainly by renal excretion with a fraction excreted unchanged[6,7].

Several physicochemical methods were found in the literature for the assay of levetiracetam in biological fluids, most of them are based on LC-MS[8] and HPLC[9-13]. Existing analytical methods reveal that little attention was paid in developing visible spectrophotometric methods by exploring thoroughly the analytically useful functional groups in levetiracetam which prompted the authors to carry out in this accord.

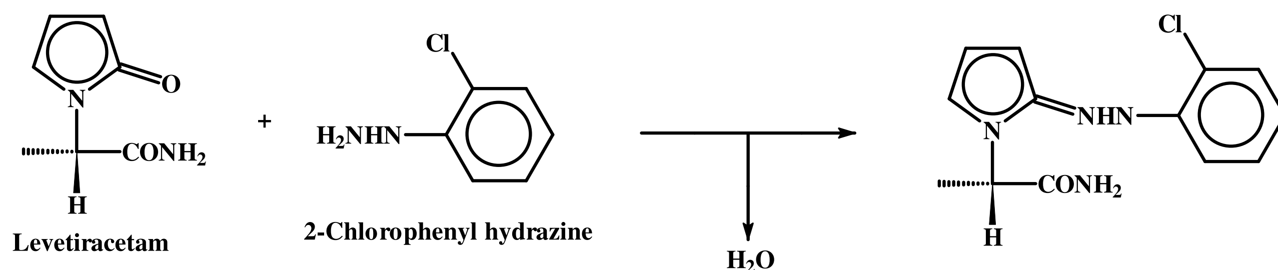
EXPERIMENTAL

All spectral measurements were made on ELICO [SL-159]-UV-visible spectrophotometer, with a pair of 1 cm matched quartz cells. All chemicals used were of analytical purity grade and all solutions were prepared in distilled water. A stock solution (1 mg/mL) of the drug was prepared by dissolving 100 mg of levetiracetam in purified water and diluting to 100 mL with purified water. Five milliliters of the stock solution was further diluted to 100 mL to obtain 50 µg/mL working standard solution. Anthranilic acid (Loba; 0.25 %) was prepared by dissolving 0.25 g of anthranilic acid in 100 mL of methanol, 2-chlorophenylhydrazine (Loba; 0.25 %) was prepared by dissolving 0.25 g of 2-chlorophenyl hydrazine in 100 mL of methanol.

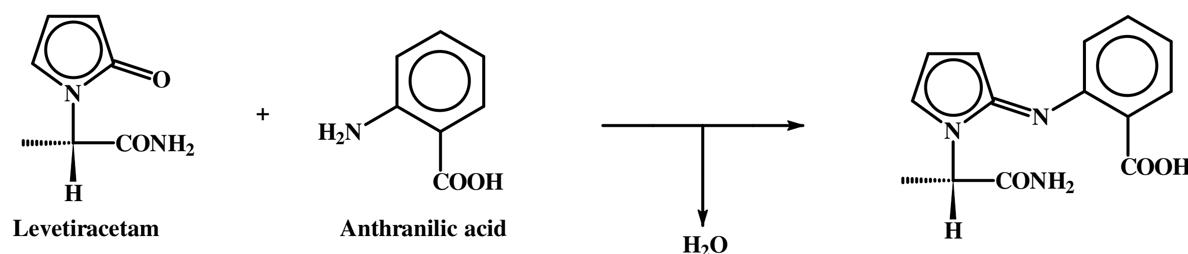
Procedure

Method A: Delivered aliquots of working standard solution of levetiracetam (0.5 mL-2.5 mL, 50 µg/mL) into a series of 10 mL volumetric flasks and 2 mL of 2-chlorophenylhydrazine was added to each of the above aliquots followed by one drop of concentrated hydrochloric acid and heated to 50-55 ºC for colour development. The absorbance of the colour derivatives were measured at 560 nm against reagent blank. The amount of levetiracetam was computed from its calibration graph.

Method-B: Different aliquots of standard solution of levetiracetam (0.5-2.5 mL,50 µg/mL) was delivered into a series of 10 mL volumetric flasks and 2 mL (0.25 %) of anthranilic acid was added to each of the above aliquots followed by one drop of concentrated hydrochloric acid and heated to 50-55 ºC for colour development. The absorbance of the colour derivatives



Scheme-I



Scheme-II

were measured at 485 nm for anthranilic acid against reagent blank. The amount of levetiracetam was computed from its calibration graph.

Assay of levetiracetam in pharmaceutical formulations: Two portions of injection powder equivalent to 50 mg of levetiracetam were weighed accurately and separately extracted into 50 mL of chloroform with shaking and the residues were filtered using Whatman No. 42 filter paper. The filtrates were evaporated to dryness under vacuum and the corresponding residues were dissolved in methanol and transferred to 50 mL standard volumetric flasks and diluted to volume with their corresponding solvents. The assay was completed following the recommended procedures for determination of levetiracetam. Commercial formulations containing levetiracetam were successfully analyzed and recovery studies were conducted by analyzing each pharmaceutical formulation by the proposed method and the results are reported in Table-2.

RESULTS AND DISCUSSION

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table-1. Regression analyses using the method of least squares were made to find slope (b), intercept (a) and correlation coefficient (r) and the results are summarized in Table-1. The per cent relative standard deviation and per cent range of error were calculated from the six measurements 3/4 of the upper Beer's law limits of levetiracetam and the results are given in Table-1. The results showed that these methods have reasonable precision. Comparison of the results obtained with the proposed and UV methods for dosage forms (Table-2) confirm the suitability of these methods for pharmaceutical dosage forms. In order to justify the reliability and suitability of the proposed methods, known quantities of pure levetiracetam was added to its various preanalyzed formulations and the mixture were analyzed by the proposed methods. The results of recovery experiments were analyzed by the proposed methods the results of recovery experiments are also summarized in Table-2. The other active in gradients and excipients usually present in pharmaceutical dosage forms did not interfere.

TABLE-1
OPTICAL CHARACTERISTICS AND PRECISION

Optical characteristics	**Method-A**	**Method-B**
λ_{max} (nm)	560	485
Beer's law limits (µg/mL)	2.5-12.5	2.5-12.5
Molar Absorptivity (mol^{-1} cm^{-1})	1.130×10^4	7.110×10^4
Correlation coefficient (γ)	0.9999	0.9998
Sandell's sensitivity (µg/cm^2/0.01 absorbance unit)	0.03546	0.03708
Regression equation (y = a + bc)		
(i) Slope (b)	0.0440	0.0240
(ii) Intercept (a)	0.0016	0.0022
Relative standard deviation (%)*	0.5239	0.9566
Percentage of range error (confidence limit)		
0.05LVCel	0.5499	1.004
0.01LVCel	0.8624	1.575

*Average of six determinations considered.

Chemistry of the coloured products: The keto group of the levetiracetam reacts with the amino group of the 2-chlorophenyl hydrazine (**method-A**)/anthranilic acid (**method-B**) resulting the formation of coloured species. The coloured species formation may be given in **Schemes I** and **II**.

TABLE-2
ASSAYOF LEVETIRACETAM IN PHARMACEUTICAL FORMULATIONS

Formulations*	Amount taken (mg)	Amount found by proposed Methods**		Reference method	Percentage recovery by proposed Methods***	
INJ-I	40	39.57	39.07	39.88	99.51 ± 0.35	99.39 ± 0.12
INJ-II	40	38.99	39.03	39.78	99.42 ± 0.18	99.69 ± 0.26

Conclusion

The proposed methods are found to be simple, sensitive selective, accurate and economical when compared to quantitative methods by HPLC and LC-MS. It can be used for the determination of levetiracetam in bulk drug and its pharmaceutical dosage forms in a routine manner.

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