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Simultaneous Estimation of Levetiracetam and its Preservatives In Oral Liquid Dosage form by Rp-Hplc Method

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Abstract

A rapid, simple, sensitive and accurate RP-HPLC method was developed and validated for simultaneous estimation of Levetiracetam and its preservatives (Methyl paraben and Propyl paraben) in oral liquid dosage form. The chromatographic separations were achieved on a Zorbax CN column (250×4.6 mm inner diameter, 5µm) using a mobile phase consisting of orthophosphoric acid buffer (pH 4.6) – acetonitrile (75:25 v/v) at a flow rate of 1.0 mL/min with UV detection at 210nm. This system produced sharp peaks with good resolution, minimum tailing and satisfactory retention time for Levetiracetam, Methyl paraben and Propyl paraben were found to be 4.601, 9.905 and 19.291 respectively.

The method was validated as per USP guidelines which include accuracy, precision, linearity and range, robustness specificity and ruggedness. The described method was linear over the range of 30-90 µg/ml for Levetiracetam with r^2 of 0.9999 and 60-180 µg/ml for Methyl paraben with r^2 0.9997 and 20-60 µg/ml for Propyl paraben with r^2 0.9999 . The average recovery of the method was 98.2%, 99.3% and 98.7% for Levetiracetam, Methyl paraben and Propyl paraben respectively.

The developed method is repeatable and selective for the analysis of Levetiracetam and its preservatives in oral liquid dosage form. Hence the method could be successfully applied for routine analysis. The developed method could also applicable in quality control testing in analysis of oral liquid dosage form and in process testing.

Keywords: Levetiracetam; RP-HPLC; Oral Liquid Dosage Form; Preservatives

Introduction

Levetiracetam (LEV) is a novel anti-epileptic agent. The chemical name of LEV, a single enantiomer is (-)-(S)-α-ethyl-2-oxo-1-pyrrolidine acetamide. [1] The chemical structure of LEV is shown in Figure 1. Its molecular formula is $C_8H_{14}N_2O_2$ and its molecular weight is 170.21. It is chemically unrelated with existing anti-epileptic drugs, differing in structure and pharmacology [2, 3]. This is a structural analogue of piracetam, which binds to a synaptic vesicle protein SV2A and is believed to impede nerve conduction across synapses. The exact mechanism by which Levetiracetam shows its antiepileptic effect is still unknown. However, it is believed that it binds to a synaptic vesicle protein, thus slowing down nerve conduction across synapses [4]. Levetiracetam is approved by the U.S. Food and Drug Administration as an adjunct in partial on set seizures, myoclonic seizures and primary generalized tonic-clonic seizures and mono therapy for partial seizures with or without secondary generalization. Levetiracetam has possible benefits for other psychiatric and neurologic conditions4 such as Tourette syndrome, autism, and anxiety disorders [4, 5].

The extensive literature survey revealed few methods are developed to estimate the drug Levetiracetam in raw material, tablets and in biological fluids using UV [5-7], RP-HPLC [2,8-18], LC-MS [19-21], Capillary electrophoresis [22], UPLC [23,24], Gas Chromatography [25,26] and HPTLC [27]. However, there is no method developed yet for the simultaneous estimation of Levetiracetam and its preservatives by RP-HPLC in oral liquid dosage forms. Hence, the present work is aimed to develop a new method for Levetiracetam and its preservatives (Methyl paraben and Propyl paraben) by RP-HPLC in oral liquid dosage form and validation of the developed method.

Experimental methods

Chemicals and Reagents

Levetiracetam standard drug, Methyl Paraben and Propyl Paraben were obtained as gift samples from Hetero research

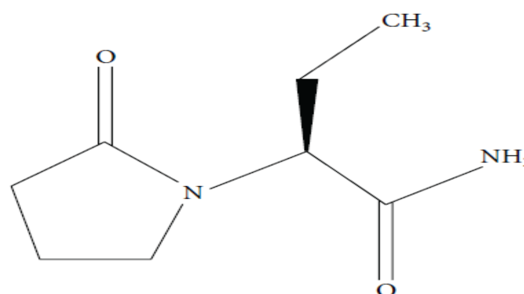

Figure 1: Chemical structure of Levetiracetam.

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labs. Formulation Keppra-1 ml oral solution was purchased from local market. Acetonitrile, Water and Methanol (HPLC grade) from Merck, orthophosphoric acid (AR) from S.D.Fine chemicals were used.

Instruments

Waters HPLC system equipped with waters separation module 2695 and auto sampler was employed for analysis. The chromatographic data was acquired by using empower 2 software. Zorbax CN, (250×4.6mm), 5μm column was used for separation. Lab India UV 3000/ UV win- Double beam UV-VISIBLE Spectrophotometer, Ultra sonicator- MAN-009-Systronics, Gelman science vaccum lamp, pH meter- μ pH system 361- Systronics were also used.

Selection of Wave Length

In setting up the conditions for development of the assay method, the choice of detection was based on the scanned absorption spectrum for Levetiracetam, Methyl paraben (MP) and Propyl paraben (PP). The U.V spectrum of Levetiracetam, Methyl paraben and Propyl paraben (Figure 2a, 2b, 2c) were obtained separately by scanning the sample solution in methanol over the wavelength range 200-400 nm against the blank**.**

HPLC Conditions

A mixture of orthophosphoric acid buffer (pH 4.6) and acetonitrile (75:25, v/v) was employed as a mobile phase. Flow rate 1.0 mL/min, injection volume 20 μl and the Detection wavelength was 210 nm.

Standard Solutions

Stock standard solution was prepared by dissolving 50 mg Levetiracetam, 50 mg Methyl paraben and 50 mg propyl paraben separately in 100 ml mobile phase.

Working standard solution was prepared to get a final concentration of 60 μg/ml Levetiracetam, 120 μg/ml Methyl paraben and 40 μg/ml propyl paraben.

Sample Solution

Weigh and transfer accurately about 5ml of Levetiracetam oral solution into 100ml clean dry volumetric flask. Dissolve and dilute to volume with mobile phase and filter. Transfer 2ml of above filtered solution into 100 ml volumetric flask and dilute to volume with mobile phase and mix.

Procedure for Estimation

20μl of blank, placebo, standard preparation (5times), and sample preparation were injected into the chromatographic system separately. Chromatogram was recorded and peak responses were measured. The placebo chromatogram was examined for any extraneous peaks observed in the chromatogram of sample preparation. The concentration of drug was calculated by using the following formula.

Concentration of drug **=**

$$\frac{A_T}{A_s} X \frac{W_S}{100} X \frac{5}{100} X \frac{100}{W_T} X \frac{50}{5} X \frac{P}{100} X A$$

Where

A_T = Area count of Drug peak in sample solution.

A_S = Average area count of five replicate injections for drug

W_S = Weight of drug working standard taken in mg

W_T= Weight of sample taken in mg

P = Purity of drug working standard used

A = weight per ml of solution.

Results and Discussion

Method Development and Optimization of HPLC Conditions

The HPLC conditions were optimized by using trials with different columns, several mobile phase compositions, flow rate and pH. The mobile phase containing orthophosphoric acid buffer (pH 4.6) and acetonitrile (75:25, v/v), Zorbax CN (250×4.6mm) 5μm column at a flow rate of 1.0 ml/minute and 210 nm wavelength detection was selected because it gave sharp peaks with good resolution, minimum tailing and satisfactory retention time.

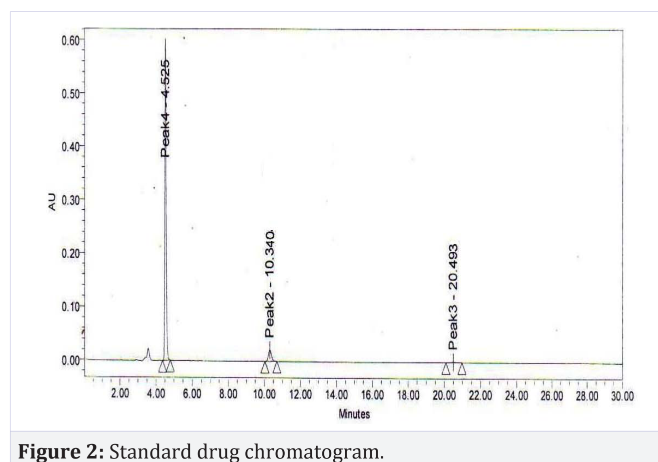

Figure 2: Standard drug chromatogram.

Method Validation

The validation of the current method was performed which include accuracy, precision, linearity and range, robustness, specificity and ruggedness [28-32].

System Suitability

The system suitability parameters were evaluated with the help of standard chromatogram which is shown in Figure 2. Plate counts (N), Tailing factor (T) and resolution (R_s) are determined from replicate injection of standard compared with method specification Levetiracetam and its preservatives solutions were prepared and injected. All the system suitability parameters were within the acceptance criteria, which is listed in Table 1.

Accuracy

Accuracy is determined by applying the method in triplicate to sample to which known amounts of analyte have been added

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at about 50%, 100% & 150% of the test concentration, i.e. (30, 60 and 90 µg/mL) of LEV, (60, 120 and 180 µg/mL) of MP and (20, 40 and 60 µg/mL) of PP. The recovery studies were carried out with three injections in each concentration. The accuracy is then calculated from the test results as the percentage of analyte recovered by the assay which is presented in Table 2.

Precision

The precision of the developed method was studied by performing intraday and interday variations. Intraday variation was determined by six replicate injections of standard in the same day and the interday was determined in six consecutive days. The precision was evaluated by calculating the %RSD of peak areas of six replicate injections of LEV, MP and PP. The % RSD of intraday precision was found to be 0.54, 0.55 and 0.81 for LEV, MP and PP respectively and the % RSD of interday precision was found to be 0.68, 0.74 and 0.98 for LEV, MP and PP respectively. The lower % RSD values showed excellent precision of the method.

Linearity

Five different concentrations of standard solutions were selected to demonstrate the linearity of the method, i.e. 30-90 µg/mL for LEV, 60-180 µg/mL for MP and 20-60 µg/mL for PP. The calibration curve was plotted using concentration versus peak area. An excellent linearity was obtained with correlation coefficient value 0.9999, 0.9997 and 0.9999 for LEV, MP and PP respectively.

Robustness

The robustness was evaluated by making slight variations in the optimized conditions such as mobile phase composition, pH of mobile phase, flow rate and temperature. The results obtained (Listed in Table 3) after the small deliberate changes in the method parameters has proven that the developed method is robust.

Specificity

The specificity of the proposed method was demonstrated by interference study. It was found that presence of some common exicipients did not cause any interferences at the retention time of LEV, MP and PP (Figure 3). Thus the developed method can be successfully applied for simultaneous determination of LEV, MP and PP in oral liquid dosage form.

Ruggedness

Ruggedness (Intermediate precision) of the method was evaluated by analyzing six samples by two analysts in the same laboratory by using different HPLC systems. From the Ruggedness data, the %RSD of the peak area of LEV, MP and PP were found to be 0.67, 0.74, 0.98 and 0.53, 0.55, 0.81 for the analyst 1 and 2 respectively, which is well within the acceptance criteria indicating that the present method is rugged.

LOD and LOQ

LOD and LOQ were calculated by using the standard deviation of the regression line of the calibration curve and its slope. The

Table1: System suitability parameters.

Parameters	Levetiracetam	Methyl Paraben	Propyl Paraben
Resolution		4.5	8.5
Tailing factor	1.06	1.28	1.1
Number of theoretical plates	13664	17740	18436
Retention time (min)	4.68	9.95	19.29

Each value is average of six determinations (n=6)

Table 2: Accuracy data (Analyte recovery study)

% of drug added	Spiked conc. (µg/mL)	*Recovered conc. (µg/mL)	*% Recovery	*% RSD
Levetiracetam 50%	30	29.41	98.06	0.469
Levetiracetam 100%	60	59.08	98.46	0.213
Levetiracetam 150%	90	88.21	98.09	0.163
Methyl Paraben 50%	60	59.41	99.03	0.162
Methyl Paraben 100%	120	119.56	99.66	0.04
Methyl Paraben 150%	180	178.34	99.08	0.161
Propyl Paraben 50%	20	19.70	98.49	0.538
Propyl Paraben 100%	40	39.48	98.69	0.375
Propyl Paraben 150%	60	59.39	98.99	0.222

*Mean of three replicates

Table 3: Robustness of the proposed method

Factor	Level	Peak area, %RSD (n=3)		
		Levetiracetam	Methyl paraben	Propyl paraben
Ratio of acetonitrile in the mobile phase	23	3276946, 0.656	3450731, 0.772	273318, 0.909
	25	3087346, 0.839	3241701, 0.828	261227, 0.882
	27	2799809, 1.201	2866337, 1.039	236391, 1.474
pH of the mobile phase	4.4	3281432, 0.472	3678641, 0.462	270626, 0.534
	4.6	3087346, 0.839	3241701, 0.828	261227, 0.882
	4.8	3074448, 1.028	2911662, 1.089	248484, 1.070
Flow rate	0.8	3281623, 0.493	3515152, 0.467	304380, 0.544
	1.0	3087346, 0.839	3241701, 0.828	261227, 0.882
	1.2	2777980, 1.16	2703662, 1.26	232058, 1.31
Temperature	25ºC	3368998, 0.595	3636370, 0.599	256101, 0.624
	Ambient	3087346, 0.839	3241701, 0.828	261227, 0.882
	30ºC	2653982, 1.283	2870714, 1.130	2870714, 1.130

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LOD values were found to be 0.937, 1.612, 0.077μg/mL for LEV, MP and PP respectively. The LOQ values were found to be 2.841, 4,887, 0.234μg/mL for LEV, MP and PP respectively.

Application of the Method for Estimation of LEV in Marketed Oral Liquid Dosage Form

Using the proposed HPLC method, the determination of LEV in its commercial oral liquid dosage form was carried out. Satisfactory results were obtained, which are in good agreement with the label claim. The results are shown in Table 4.

Conclusion

A simple, specific, reliable method was developed for simultaneous estimation of Levetiracetam and its preservatives in marketed oral liquid dosage form. The method offers good resolution between the proposed components within a suitable analysis time. Based on the results obtained, it was found that the developed method is accurate, precise and sensitive. The proposed method has an advantage over the published methods for analyzing Levetiracetam in presence of the preservatives used in commercial formulation. Therefore the applied method could be conveniently adopted for the routine quality control analysis of Levetiracetam and its preservatives from its oral liquid dosage form.

Table 4: Application of developed method for assay of marketed formulation

Drug	Label claim	Injection No	Peak Area	Amount found (mg/ mL)	% Assay	Mean % Assay	%RSD
Levetiracetam oral Solution	100 mg/ mL	1	3089267	99.97	100.06	100.02	0.041
		2	3086798	99.89	99.98		

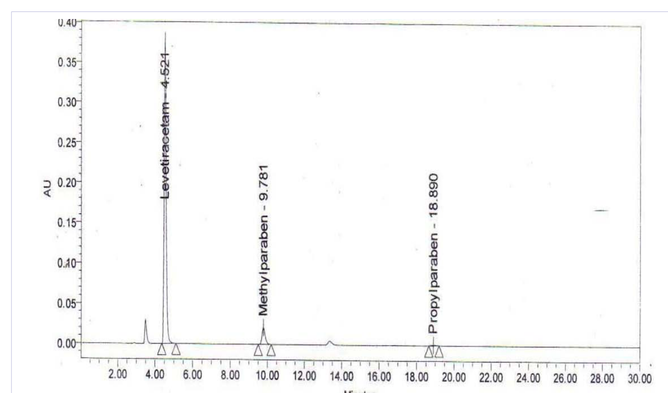

Figure 3: Chromatogram for Specificity.

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