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Simultaneous Determination of Levetiracetam and Preservatives in Oral Solution Formulation Using Hplc-Uv Method with a Programble Detection Wavelength

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Abstract: A reversed-phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous determination oflevetiracetam, methylparaben and propylparaben in levetiracetam oral solution formulation. The separation was achieved on Equisil BDS, C18, 5 µm, (150 mm × 4.6 mm) using 1.4 g/L of NaH_2PO_4 : Methanol in ratio (55:45) respectively pH 7.7 by NaOH as mobile phase and at a flow rate of1.0 mL/min. Detection was carried out using a UV detector Start with 240 nm then at 9 minutes change to 254 nm. The total chromatographic analysis time per samplewas about 14min. Analytical parameters system suitability, specificity, linearity, precision, repeatability accuracy, LOD/LOQ and stability of standard solution were determined by validation procedure and found to be satisfactory.

Keywords: Levetiracetam / Methylparaben / Propylparaben / HPLC-UV.

INTRODUCTION

Levetiracetam (Fig.1) is a novel antiepileptic agent; with an IUPAC or systemic name of (2S)-2-(2-Oxopyrrolidin-1-yl)butanamide[1]. It is used as an adjunctive therapy in the treatment of partialseizures[2]. Levetiracetam can prevent myoclonic jerks and generalizes epileptiform activity in patients with photosensitive epilepsy[3]. It is also used in veterinary medicine for similar purpose[4]. It is also used to treat neuropathic pain[5]. The bioavailability of Levetiracetam after oral administration, is almost equal to 100%[6]. The biotransformation occurs by the enzymatic hydrolysis of acetamide group[7]. The metabolized drug is excreted through urine[6].

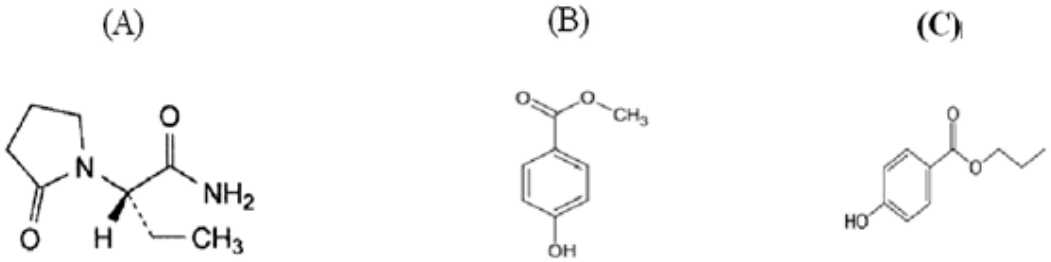

Figure 1: Structure of Levetiracetam(A), Methylparaben (B) and Propylparaben(C)

Pharmaceutical preparations which need an aqueous vehicle such as syrups and powders fororal suspensions require safeguards from microbial contamination[8], which may affect product stability or infect the consumers. This is accomplished by the addition of anti-microbialagents in the formulation to destroy and inhibits the growth of those organisms that may contaminate the product during manufacture or use[7]. The choices of the preservatives are limited, which are generally effective to control mouldand yeast growth.

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These include p-hydroxybenzoic acid esters: methyl paraben (M.P.)$C_6H_4(OH)COOCH_3$(Fig-1B)and propyl paraben (P.P.) $C_6H_4(OH)COOC_3H_7$(Fig-1C), which are most commonly used to control bacterial growth due to their broad antimicrobial spectrum with good stability and non-volatility[9]. M.P and P.P. are usually used in combination as they possess a synergistic activity when used together[10].

Several types of analytical procedures have been proposed for the analysis of Levetiracetam in pharmaceutical formulations; RP-HPLC-using gradient elution Method have been developedforthe simultaneous determination of Methyl and Propyl Parabens with Levetiracetam in Pure Form and Pharmaceutical Formulation [10].A RP-HPLC method has been developed for simultaneous determination of co-administered levetiracetam and pyridoxine HCl in prepared tablets using BDS Hypersil C8 (250 × 4.6 mm, 5 µm) column applying an isocratic mobile phase containing methanol and 25 mM KH_2PO_4 buffer pH 3 (38.4:61.6, v/v) at 0.8 mL/min flow rate with UV detection at 214 nm and 5 µL injection volume [11]. A HPLC-UV and its identification by LC-ESI-MS a quantitative determination of levetiracetam in human urine has been developed [12].A gas chromatography Method has been developed for the Quantitative determination of levetiracetam by using ethyl chloroformate as a derivatizing reagent in pure and pharmaceutical preparation [13]. An Ultra-High-Performance Liquid Chromatography–Photodiode Absorbance has been developed for the determination of ng/mL Levetiracetam[14]. A HPLC-diode array detection method was developed and validated to simultaneously quantify lacosamide, levetiracetam and zonisamide in human plasma to implement pharmacokinetic drug monitoring and individualize the posology of the antiepileptic drugs [15]. An Ultra-Performance Liquid Chromatography–Tandem Mass Spectrometry Method for the Concurrent has been developmed and Validated for Measurement of Gabapentin, Lamotrigine, Levetiracetam, Monohydroxy Derivative of Oxcarbazepine, and Zonisamide Concentrations in Serum in a Clinical Setting[16].A spectrophotometric method has been developed for the determination of levetiracetam in pharmaceutical formulations using molybdenum blue method was developed byprepairing stable and intense blue colored molybdenum blue complex[17]. A Spectrophotometric method has been developed for the determination of Levetiracetam by Developing Coloured Complexes with 2-Chlorophenylhydrazine and Anthranilic Acid [18].A HPLC method has been developed for the determination of preservative parabens in oral and injection formulations by hplc[19]. A New Validated HPLC Method for the Simultaneous has been developed for the determination of 2-phenoxyethanol, Methylparaben, Ethylparaben and Propylparaben in a Pharmaceutical Gel[20]. A High Performance Liquid Chromatography method has been developed for the quantitative Analysis of Methyl and Propylparabebny[10, 21-26].

The aim of this work is to develop and validate a simple method for determination of LEV, M.P and P.P in bulk and combined dosage form and to overcome the concentration difference (10:1) and response factor difference between M.P and P.P[27].

MATERIALS AND METHOD

Reagents and Chemicals

Levetiracetam (LEV) (USP R.S),Methylparaben (M.P) and Propylparaben (P.P) were obtained from Salicylates & Chemicals Pvt Ltd, (Mumbai, India). Keppra 100mg/ml® oral solution were purchased from the local market. Methanol (HPLC gradeoptained from Scharlau), NaH_2PO_4 and Sodium hydroxide were obtained from Panreac, Sample solution filter were purchased from Whatman for syringe filter. Ultra pure water (Milli-Q) (Millipore Corporation, Billerica, MA, USA) was used.

Instrumentation and Chromatographic Conditions

1) HPLC-UV Analyses

The HPLC system (Waters, USA) was equipped with autosampler, Binary HPLC Pumps, Dual lamb Absorbance Detector and In-Line Degasser ISA Card. Data acquisition was performed on Empower software. The detector was set to Start with 240 nm then at 9 minutes change to 254 nm. The HPLC separation and quantitation were achieved on Equisil BDS, C18, 5 µm, (150 mm × 4.6 mm) analytical column (Dr. Maisch HPLC GmbH Germany). All determinations were performed at 30 ºC. The mobile phase was 1.4 g/L of NaH_2PO_4 : Methanol in ratio (55:45) respectively pH 7.7 by NaOH, which was run Isocratic. Flow rate was 1.0 ml/min and injection volume was 20 µl. The diluent: Methanol: water (20:80).

Preparation of Standard Solutions

P.P. and M.P. stock solution was prepared by transferring accurately weighed about25 mg of Propylparaben standard and 250 mg of methylparaben standard to a 50 ml volumetric flask, dissolving in 30 ml diluent and completing to volume with the diluent.

LEV,P.P and M.P standard solution was prepared by transferring accurately weighed about 500 mg of Levetiracetam standard to a 100 ml volumetric flask; adding 50 ml of diluent. Dissolving, adding 2 ml of P.P. and M.P. stock solution then Completing to volume with the diluent.

Preparation of Sample Solutions

5 ml of the Keppra 100mg/ml® oral solution was accurately measured and transferred to a 100 mL volumetric flask and dissolved in 100 ml diluent in an ultrasonic bath for 10 min and filtered through 0.45 µm membrane filter.

Validation

The method was validated in accordance with the ICH requirements[28], with respect tosuitability, specificity, precision, linearity, accuracy, repeatability, ruggedness and stability of standard solution

1) System suitability

To ensure the validity of the analytical procedure, a system suitability test was carried out. Data from six injections of 20 µL of the working standard solution were used for evaluating the system suitability parameters, such as retention time, resolution theoretical plates, asymmetry.

2) Selectivity

The ability of an analytical method to unequivocally assess the analyte in the presence of other components can be demonstrated by evaluating specificity.

To evaluate the method selectivity the excipients used for Levetiracetam oral solution®without LEV, P.P and M.p were injected. Samples were prepared as described above, to ensure the identity of the target analyte.

3) Linearity

Linearity is studied to determine the range over which analyte response is a linear function of concentration. This study was performed by preparing standard solutions at five different concentrations (50, 80, 100, 120 and 150) % of the single dose concentration which is 5000, 100 and 10 µg mL^{-1} of LEV, M.P and P.P. analyses were performed in triplicate. The calibration curves were constructed by plotting the peak areas against concentrations.

4) Limits of detection and Limit of Quantitation

The detection limit of an individual analytical procedure LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

The quantification limit of an individual analytical procedure LOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantification limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Based on the Standard Deviation of the Response and the Slope Following formulae were used;

$$LOD = 3.3\times/S$$
$$LOQ = 10\times/S$$

Where, is the standard deviation of the response at low concentrations and S is the slope of the calibration curve .

5) Precision

The precision of an analytical method is the closeness of replicate results obtained from analysis of the same homogeneous sample. Precision was considered at two levels, i.e. repeatability and intermediate precision, in accordance with ICH recommendations[28].

Repeatability

Repeatability was determined by performing six analyses at three concentrations on the same day.

6) Ruggedness

The ruggedness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage such as day to day and analyst to analyst. The ruggedness of the method was tested by analysis of the same sample in triplicate under a variety of test conditions such as different days, analysts.

7) Accuracy

The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. It is expressed as recovery (%), which is determined by the spiking test method. 80% ,100% and 120% of a targeting concentration (5000µg mL-1 for LEV, 10µg mL-1 for P.P and 100 µg mL-1 for M.P) was spiked . The experiment was performed in triplicate. The peak areas were used to calculate means, RSD% and % recovery.

8) Stability of Standard Solution

The solution stability of standard solution was carried out by leaving the

standard solution at concentration of 5000 µg mL-1 for LEV, 10µg mL-1 for P.P and 100 µg mL-1 for M.P in the injector glasses at room temperature for more than 24hour. The same sample solutions were injected at different time interval up to the study period against freshly prepared solutions.

RESULTS AND DISCUSSION

Method Development

An Equisil BDS, C18, 5 µm, (150 mm × 4.6 mm) analytical column (Dr. Maisch HPLC GmbH Germany), maintained at (25 ºC) was used for the separation of LEV, P.P and P.P. The mobile phase was 1.4 g/L of NaH_2PO_4 : Methanol in ratio (55:45) respectively pH 7.7 by NaOH, which was run Isocratic. Flow rate was 1.0 ml/min and injection volume was 20 µl. The diluent: Methanol: water (20:80).The method was validated for the determination of LEV, P.P and P.P in Levetiracetam oral solution. Under the proposed chromatographic conditions, all peaks were chromatographically resolved Figure 2.

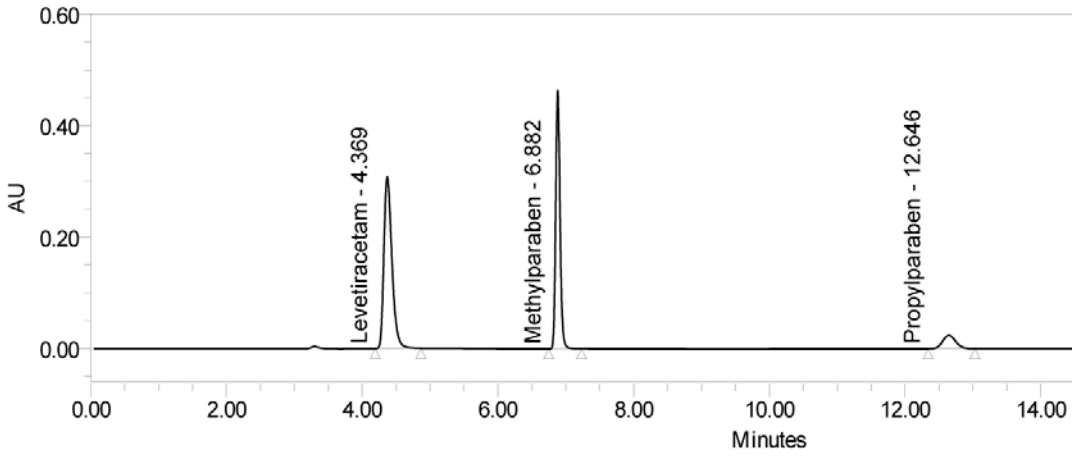

Figure 2: Chromatogram shows the standard solution of LEV, M.P and P.P

Method Validation

The developed method was validated according to the ICH guidelines[28], for the following parameters: system suitability, specificity, linearity, LOD/LOQ, repeatability Ruggdness, accuracy, and stability of standard solution.

1) System Suitability

To ensure the validity of the analytical procedure, a system suitability test was carried out. Data from six injections of 20 µL of the working standard solution were used for evaluating the system suitability parameters, such as retention time, capacity factor, resolution theoretical plates, asymmetry, and selectivity. Results showed in Table (1).

Table 1: System suitability parameters

Parameters	LEV	M.P	P.P	Acceptance criteria
Asymmetry	< 1.4	< 1.2	< 1.2	≤ 2
Resolution		> 14	> 24	> 2
Theoretical plates	>6600	>56000	> 20000	> 2000

2) Selectivity

Selectivity is the ability of the proposed method to accurately determine the analytes in the presence of other matrix components.

The analysis of the placebo solution composed of excipient mixture showed no interference with the target analytes. Overall, these data confirmed that presence of excipients did not interfere with the analysis, indicating selectivity of the method.

3) Linearity

Different concentrations for LEV, P.P and M.P of the mixture of three analytes were prepared for linearity studies. A typical HPLC chromatogram obtained during simultaneous determination of LEV, P.P and M.P is given.

The calibration curves obtained by plotting peak area against concentration showed linear relationship over a concentration range of 2500-7500 µg mL^{-1} for LEV, 5 -15 µg mL^{-1} for P.P and 50-150 µg mL^{-1} for M.P. The linear regression coefficient values (R^2) were found

The method was found to be linear, as the square of correlation coefficient (r) is greater than 0.999 for LEV, P.P and M.P, indicating a high degree of linearity. Results of linearity of the proposed HPLC method are summarized in Table (2).

LOD was found to be 19.5, 0.003 and 0.25µg mL^{-1} for LEV, P.P and M.P, respectively. LOQ was found to be 59.1, 0.009, 0.75µg/mL for LEV, P.P and M.P, respectively. Small values of LOD and LOQ indicate high sensitivity of the proposed method. Regression characteristics of the proposed HPLC method are summarized in Table (2).

Table 2: Five level calibration graphs for LEV, M.P and P.P

Analyte	Range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	Slope	Intercept	r^2
LEV	2500-7500	19.5	59.5	519.12	65053.06	0.9997
M.P	50-150	0.25	0.75	20492.41	-11822.35	0.9998
P.P	5-15	0.003	0.009	32536.6	-1173.41	0.9998

4) Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical method is usually expressed as the relative standard deviation of a series of measurements. Precision may be a measure of either the degree of reproducibility or repeatability of the analytical method under normal operating conditions. Standard solution was prepared according to the description of the method. Standard solution was injected six times from the same vial to calculate system precision. The results were tabulated and the percentage relative standard deviation was calculated. The results are shown in the table (3) and (4).

Table 3: Inter and intra-day precision (%RSD) data for **targeting concentration (**2500-750 µg mL^{-1} for LEV, 5 -15 µg mL^{-1} for P.P and 50-150 µg mL^{-1} for M.P)

Analyte	Concentration
	100 %
LEV	1.65
M.P	1.32
P.P	1.18

Table 4: Analyst to analyst precision (%RSD) data for **targeting concentration (**2500-750 µg mL^{-1} for LEV, 5 -15 µg mL^{-1} for P.P and 50-150 µg mL^{-1} for M.P)

Analyte	Concentration
	100 %
LEV	1.6
M.P	1.02
P.P	1.23

The low value of % RSD obtained (< 2.0 %) showed that the precision of the system is accepted.

5) Accuracy

The accuracy of the HPLC assay method was assessed by adding known amount of drug solution to a placebo solution of known concentration and subjecting the samples to the proposed HPLC method. The recovery studies were replicated 3 times.

The accuracy was expressed in terms of recovery and calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 100 so as to give the percentage recovery. The results are furnished in Table (5). recovery values demonstrated that the method was accurate within the proposed range.

Table 5: Accuracy (% recovery) data for targeting concentration (2500-750 µg mL^{-1} for LEV, 5 -15 µg mL^{-1} for P.P and 50-150 µg mL^{-1} for M.P)-

% of targeting concentration	LEV % recovery ± SD	M.P % recovery ± SD	P.P % recovery ± SD
80 %	100.60 ± 0.38	99.87 ± 0.34	100.96 ± 0.33
100 %	99.87 ± 0.34	100.18 ± 1.49	101.10 ± 0.96
120 %	99.24 ± 0.57	101.18 ± 0.65	100.16 ± 0.86

6) Stability of standard solution

The solution stability of standard solution was assessed on the same solution for about 37 hours. The results were tabulated and the percentage relative standard deviation was calculated. The results are shown in table (6). The low value of % RSD obtained (< 3.0 %) showed that the standard is stable for 37 hours.

Table 6: Stability of standard solution (%RSD) data for **concentration** 2500-750 µg mL^{-1} for LEV, 5 -15 µg mL^{-1} for P.P and 50-150 µg mL^{-1} for M.P

Analyte	Concentration
LEV	1.65
M.P	1.32
P.P	1.18

CONCLUSIONS

The proposed HPLC method was found to be simple, rapid, precise, accurate and sensitive for the determination of levetiracetam and preservatives in oral solution dosage form. Hence, this method can easily and conveniently adopt for routine analysis of levetiracetam in pure and its pharmaceutical formulations during the quality control of pharmaceutical preparation.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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