

Research Article

Development and Validation of New RP-HPLC Method for Simultaneous Determination of Methyl and Propyl Parabens with Levetiracetam in Pure Form and Pharmaceutical Formulation

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A simple and robust high-performance liquid chromatography (HPLC) method is described for the assay for levetiracetam (LTC), methyl paraben (MHB), and propyl paraben (PHB) either in their pure form or in commercial Levepsy[®] syrup. The method is selective and stability indicating and all chromatographic conditions were studied to obtain adequate separation of LTC, MHB, and PHB from their degradation products and from excipients. The HPLC separation was carried out on a RP C18 Hypersil BDS analytical column (150 mm × 4.6 mm ID) using gradient elution system. The mobile phase flow rate was 1.5 mLmin⁻¹ and the column temperature was kept at 40°C. Complete separation of the studied components was obtained within a cycle time of 8 min. LTC, MHB, and PHB were eluted at 1.56, 5.86, and 7.85 min, respectively. Detection was carried out at 240 nm using a dual wavelength detector. The method has been validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantitation, robustness, and ruggedness. The proposed method was successfully applied for the determination of LTC in the presence of parabens in Levepsy syrup.

1. Introduction

Levetiracetam (LTC) is an important antiepileptic drug that is used as a monotherapy or in combination with other drugs in patients who suffered from partial and secondary generalized seizures [1]. Despite its wide use, few HPLC procedures have been developed for LTC determination in biological fluid and in tablet formulation using either mass spectrometry or UV detection [2–7] or capillary electrophoresis [8].

Parabens are widely used as preservatives in industrial pharmacy. They are commonly used due to their bactericidal and fungicidal properties. In addition, parabens are of low cost and better efficacy over other natural alternatives like grapefruit [9]. However, parabens have been detected in breast cancer tumors (about 20 ng/g of tissue) [10]. Therefore, it is very important to determine parabens in formulations

to be sure that their concentrations do not exceed the limit specified by the International Conference on Harmonization (ICH) guidelines (ICH 2005) [11].

However, no published HPLC method was developed for the determination of parabens with levetiracetam. Therefore, the development of a robust and selective analytical HPLC method would be necessary for determination of methyl and propyl parabens with levetiracetam in either pure form or syrup as an example of pharmaceutical formulation.

2. Experimental

2.1. Materials and Reagents. Pharmaceutical grade levetiracetam (purity 99.9%) was purchased from Hetero Drugs, pharmaceutical company, India. Methyl and propyl parabens were purchased from Clariant, Switzerland. The used chemicals

in all experiments were of analytical grade. HPLC grade methanol and acetonitrile (POCH S.A., Poland), HCl, NaOH, H₂O₂, potassium dihydrogen phosphate, and orthophosphoric acid 85% (Merck, USA) were used. Fresh double distilled water was used throughout the whole experiment. Leveysy syrup (Egyptian International Pharmaceutical Industry Co., Egypt) labeled to contain 100 mg levetiracetam/mL was purchased from the local market.

2.2. Instrumentation and Chromatographic Conditions. Waters Alliance HPLC system, which consisted of a solvent management system 2695, a thermostatically controlled column apartment, a dual wavelength detector, and an autosampler, was used. Data processing and management of the HPLC system were performed using the Empower®2 software (All Waters, Millford, MA, USA).

The pH of the mobile phase was adjusted using a Metrohm 744 pH meter (Herisau, Switzerland).

HPLC separation of LTC, MHB, and PHB was achieved using reversed phase C18 Hypersil BDS analytical column (150 mm × 4.6 mm i.d., 3.5 μm particle size, Waters, Millford, MA, USA). A gradient mobile phase system composed of (A) 0.05 M phosphate buffer pH 3.5 : acetonitrile (90% : 10%) for 0–4 min and (B) 0.05 M phosphate buffer pH 3.5 : acetonitrile (40% : 60%) for 4–10 min was used.

The temperature of column was kept at 40°C. Flow rate was adjusted at 1.5 mL·min⁻¹. The injection volume was 10 μL. Detection was achieved at 240 nm. Prior to use, the mobile phases were degassed and filtered by passing through a 0.45 μm pore size membrane filter (Millipore, Milford, MA, USA).

2.3. Preparation of Stock Solutions. In a 100 mL volumetric flask, a combined standard stock solution of accurately weighted preservatives MHB (200 mg) and PHB (20 mg) was prepared in methanol.

In a 100 mL volumetric flask, a mixed standard solution of MHB, PHB, and LTC was prepared using 1 mL of the preservatives stock solution and an accurately weighted amount of LTC (100 mg). It was vortexed and completed to 100 mL with methanol. The final concentrations of analytes were 1000, 20, and 2 μgmL⁻¹ for LTC, MHB, and PHB, respectively.

2.4. Preparation of Working Samples. In 100 mL volumetric flask, 1 mL of the Leveysy syrup was transferred and then made up to 100 mL with methanol.

2.5. Construction of Calibration Curves. Aliquots of standard stock solution of LTC, MHB, and PHB were taken and adjusted with methanol to obtain their final concentrations in the range of 500–1500 μgmL⁻¹, 10–30 μgmL⁻¹, and 1–3 μgmL⁻¹ for LTC, MHB, and PHB, respectively. Calibration curves were constructed by plotting peak areas against the corresponding injected concentrations for each analyte (Table 1).

2.6. Validation Study. The developed analytical method was validated by means of system suitability, linearity, accuracy,

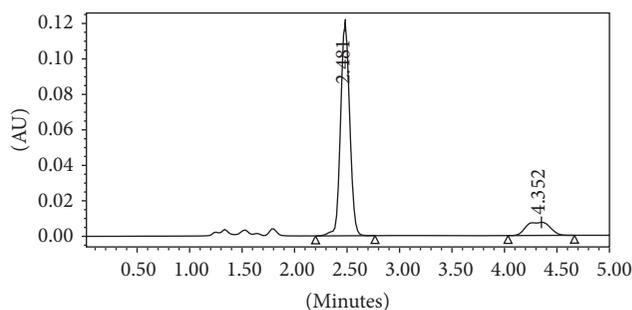


FIGURE 1: A typical chromatogram of a 10 μL injection of a standard mixture of 1000, 20, and 2 μgmL⁻¹ LTC, MHB, and PHB, respectively, by isocratic elution using Hypersil cyano analytical columns (150 mm × 4.6 mm), detection at 254 nm, and the other chromatographic conditions as optimized.

precision, specificity, limit of detection (LOD), limit of quantification (LOQ), ruggedness, and robustness as described in ICH guidelines [11].

2.7. Forced Degradation Studies of Levetiracetam. To achieve acid and base-induced degradation, 1 mL of methanolic stock solution was transferred into 100 mL volumetric flask before addition of 10 mL of 1 N HCl. Alkaline degradation study was carried out in a similar manner using 1 N NaOH. These solutions were placed in a boiling methanol bath for about 3 minutes before cooling for another 5 minutes. The contents were then neutralized by addition of 0.01 N NaOH and HCl, respectively, to reach pH ~7 and completed to 100 mL with methanol.

To carry out hydrogen peroxide-induced degradation, 1 mL of methanolic stock solution was transferred into 100 mL volumetric flask before addition of 10 mL of H₂O₂ (5 mL of 35% H₂O₂ in 100 mL H₂O). The solution was then placed in boiling methanol bath for about 3 minutes. After that, the contents were cooled for 5 minutes and then completed to 100 mL with methanol.

To achieve heat degradation, 1 mL of methanolic stock solution was transferred into 100 mL volumetric flask and 10 mL methanol was added. The solution was then placed in boiling methanol bath for about 3 minutes before cooling for 5 minutes and completing the volume to 100 mL with methanol.

3. Results and Discussion

Our proposed method allows good elution of levetiracetam, methyl paraben, and propyl paraben within a short chromatographic run time <8 min.

As the selection of the right column chemistry (bonded phase) is very important to attain optimum separation in the shortest run time, isocratic chromatographic conditions were tested using another brand of column. Hypersil cyano analytical column (150 mm × 4.6 mm) was used. However, this led to tailing and distortion of the peak shape (Figure 1).

For further optimization of the method, different wavelengths and percentages of mobile phase (phosphate buffer

TABLE 1: Regression and statistical parameters for determination of LTC, MHB, and PHB using the proposed HPLC method.

Parameter	Levetiracetam	Methyl paraben	Propyl paraben
<i>System suitability</i>			
$t_R \pm SD$ (min)	$1.561 \pm 8.22 \times 10^{-3}$	$5.855 \pm 0.6 \times 10^{-2}$	$7.844 \pm 7.8 \times 10^{-2}$
USP plate count (N)	3.36×10^3	3.59×10^4	9.04×10^4
Resolution (R_s)	—	3.7	4.45
Symmetry factor (T)	1.08	1.04	0.996
Selectivity (α)	—	4.91	1.08
<i>Linearity and regression data</i>			
Linearity range ($\mu\text{g mL}^{-1}$)	500–1500	10–30	1–3
LOD ($\mu\text{g mL}^{-1}$) ^a	0.0078	0.025	0.029
LOQ ($\mu\text{g mL}^{-1}$) ^a	0.023	0.076	0.088
Slope (b) ^b	220896	21738	31501
Intercept (a) ^b	3880	−3407	5574.4
Correlation coefficient (r^2)	0.9996	0.9999	0.9994

^aLOD: limit of detection; LOQ: limit of quantitation.

^bRegression equation for the ratio of peak area of the analyte against concentration of the analyte.

TABLE 2: Accuracy of the proposed HPLC method for determination of LTC, MHB, and PHB in Levepsy syrup.

Levetiracetam			Methyl paraben			Propyl paraben		
Nominal conc. mg mL^{-1}	Found conc. mg mL^{-1}	Recovery%	Nominal conc. $\mu\text{g mL}^{-1}$	Found conc. $\mu\text{g mL}^{-1}$	Recovery%	Nominal conc. $\mu\text{g mL}^{-1}$	Found conc. $\mu\text{g mL}^{-1}$	Recovery%
0.75	0.748	99.72	15	15.199	101.33	1.5	1.4921	99.48
1	0.995	99.51	20	20.134	100.67	2	2.0238	101.19
1.25	1.259	100.73	25	25.261	101.04	2.5	2.5122	100.49
1.5	1.495	99.69	30	29.931	99.77	3	2.9698	98.99
Mean		99.91			100.70			100.04
SD		0.56			0.68			0.99
RSD		0.557			0.672			0.989

Data are average of three experiments.

and methanol) were tested. This led to increased retention time and decreased sensitivity (data not included). Based on the above results, gradient elution improved resolution and reduced the time needed to separate relatively polar components using a C18 Hypersil BDS analytical column (150 mm \times 4.6 mm ID). Mobile phase (A) was 0.05 M phosphate buffer pH 3.5 set at 90% (with 10% acetonitrile) for 4 min. Subsequently, the percentage was decreased to 40% (with 60% acetonitrile) and remained unchanged for 6 min at detection wavelength of 240 nm. These conditions enabled the complete separation of the studied compounds in less than 8 minutes at flow rate of 1.5 mLmin^{−1}. The LTC, MHB, and PHB were eluted at 1.56, 5.86, and 7.85 min, respectively (Figure 2).

3.1. Method Validation. The results indicated good column efficiency (number of theoretical plates, N , was >2500). Selectivity was higher than one and the symmetry factor was between 0.95 and 1.15 indicating good system suitability (Table 1). The regression lines show high correlation coefficient values ($r^2 > 0.999$) as well as low LOD and LOQ values. For accuracy, our method has shown high accuracy with good recoveries of 99.91 ± 0.557 , 100.7 ± 0.672 , and 100.04 ± 0.989

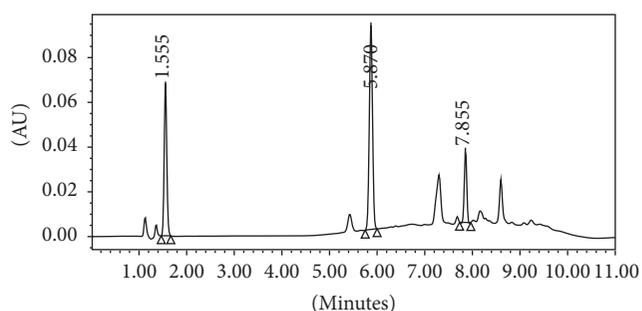
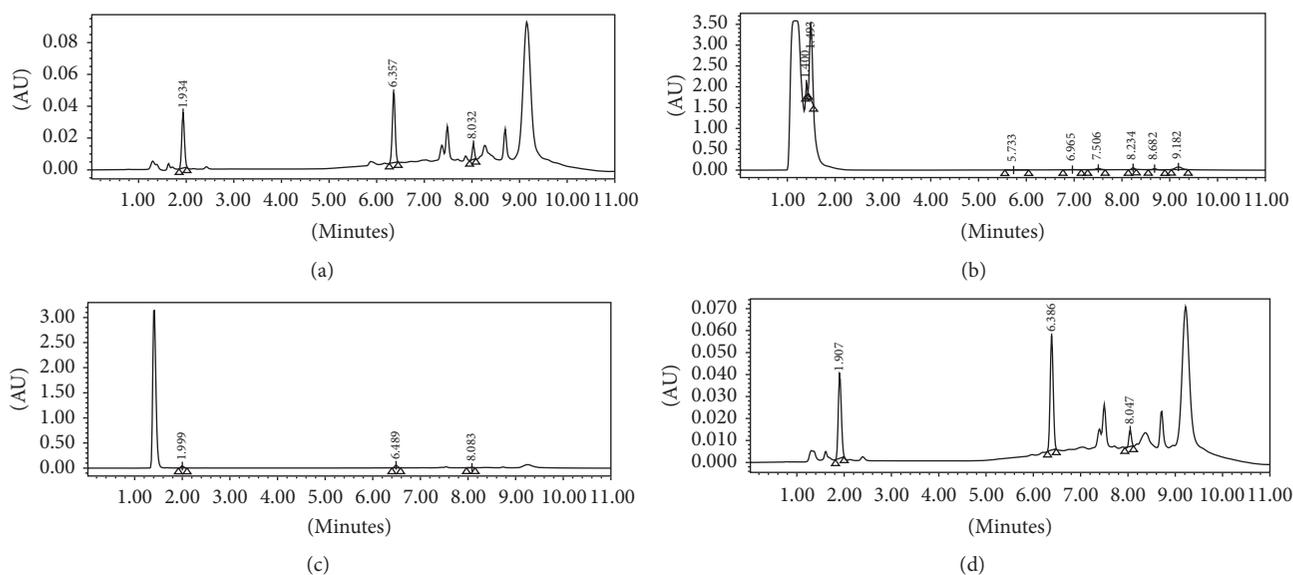


FIGURE 2: A typical chromatogram of a 10 μL injection of a standard mixture of 1000, 20, and 2 $\mu\text{g mL}^{-1}$ LTC, MHB, and PHB, respectively, at flow rate 1.5 mLmin^{−1} and detection at 240 nm.

(mean% \pm RSD) for LTC, MHB, and PHB, respectively, which are present in Levepsy syrup (Table 2, data is average of three experiments). Good precision was indicated by % RSD values lower than 1.1% for six determinations of LTC, MHB, and PHB in their standard solution (Table 3). The proposed method was found to be specific and selective as there was no interference from the other additives used in the syrup

TABLE 3: Method precision (% repeatability) of the proposed HPLC method for determination of 6 replicates of LTC, MHB, and PHB.

Nominal conc. 1 mgmL ⁻¹	Levetiracetam		Nominal conc. 20 µgmL ⁻¹	Methyl paraben		Nominal conc. 2 µgmL ⁻¹	Propyl paraben	
	Retention time	Peak area		Retention time	Peak area		Retention time	Peak area
Mean	1.56	246898		5.66	426905.7		7.850	72556.5
SD	0.004	2603.45		0.015	1263.981		0.00527	392.088
RSD	0.242	1.054		0.269	0.296		0.067	0.540

FIGURE 3: HPLC chromatogram of LTC, MHB, and PHB degradation using (a) 1N HCl, (b) 1N NaOH, (c) 35% H₂O₂, and (d) heat degradation.

formulation. In addition, the method was found to be stability indicating as the peaks of LTC, MHB, and PHB were well resolved from other degradation peaks (Figure 3).

Ruggedness of the developed method was studied by using different but typical test conditions. The different test conditions were different analysts and different days using 3 replicates of a standard sample solution (100% conc.) for each determination. Samples were analyzed by two different analysts or through two successive days. The slight variations in the examined conditions had no significant effect on the separation, shape, and symmetry of the peaks; however the retention time and peak areas slightly increased. The RSD% ($n = 3$) was less than 3% indicating acceptable ruggedness.

Robustness was checked by observing how a method stands up to slight variations in normal operating conditions such as slight variation in mobile phase composition ($\pm 1\%$) or flow rate (± 0.05 mLmin⁻¹) or using different ratio of the organic modifier and the other factors as the pH and flow rate were kept constant [11]. The obtained results indicated that there was no significant effect on peak area and retention times upon small changes in chromatographic factors with RSD% values lower than 2.5%. The method was found to be more sensitive to the slight changes in the mobile phase composition indicated by higher RSD% values ($>2.5\%$).

4. Conclusion

The developed method provides a convenient and efficient method for the determination of important parabens with levetiracetam in pure form and syrup. The complete separation of the analytes was achieved within short chromatographic run time (only 8 min) with no interfering peaks allowing the analysis of a large number of samples in a short period of time. This gives the method high interest for routine sample analysis especially as no additional extraction or separation is required.

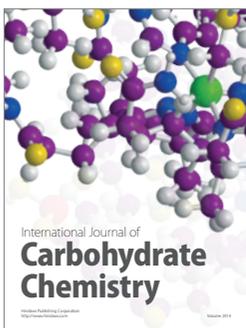
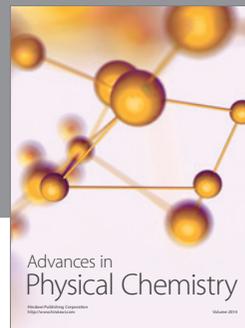
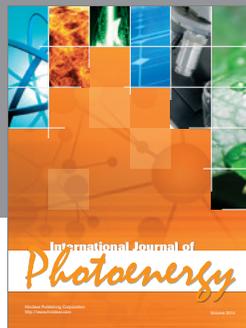
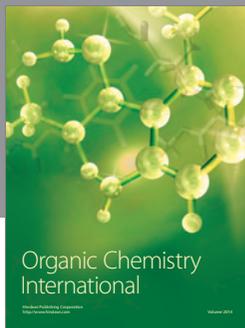
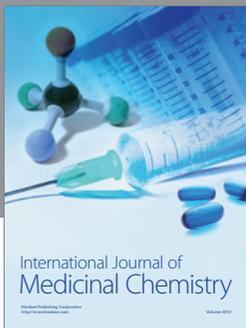
Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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