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Original Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING HPTLC METHOD FOR DETERMINATION OF LEVETIRACETAM IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: To develop a validated stability indicating HPTLC method for determination of levetiracetam in pharmaceutical dosage form.

Methods: Chromatographic separation was performed on aluminum plate precoated with Silica Gel 60 F_{254} using Toluene: Acetone: Methanol (6:2:2) v/v/) as mobile phase followed by densitometric scanning at 210 nm.

Results: The chromatographic conditions gave compact spot for levetiracetam at Rf value of 0.35 ± 0.005 and specificity in accordance with international conference on harmonization (ICH) prescribed stress conditions. The calibration curve was found to be linear in the range 500 -3000 ng/band. The limit of detection and quantitation were found to be 19.76 and 65.89 ng/band, respectively.

Conclusion: A new simple, accurate, precise and selective stability-indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for the determination of levetiracetam in pharmaceutical dosage form. The proposed method can be applicable for the routine determination of levetiracetam in bulk and formulation.

Keywords: Levetiracetam, HPTLC, Stability indicating method.

INTRODUCTION

Levetiracetam (LTC) is a new antiepileptic drug that is currently used as an add-on therapy or monotherapy in patients with partial and secondary generalized seizures [1]. Levetiracetam (Figure 1), (-)-(S)-α-ethyl-2-oxo-1-pyrrolidine acetamide, C8H14N2O2, is chemically unrelated with other antiepileptic drugs in current use, differing in structure and pharmacology [2, 3].

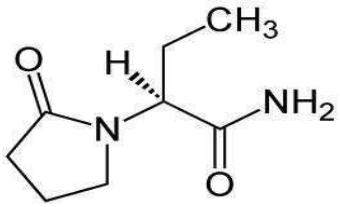


Fig. 1: Chemical structure of Levetiracetam

Literature survey revealed several HPLC methods for determination of Levetiracetam in biological fluid and in tablet formulation [4-10]. Also capillary electrophoresis method has been reported for analysis of Levetiracetam in tablets [11]. Stability indicating HPLC method has been reported for estimation of LTC [12].

But no reports were found for stability indicating High Performance Thin Layer Chromatographic (HPTLC) method for the estimation of levetiracetam in bulk and pharmaceutical formulation. Thus new simple, accurate, precise stability-indicating HPTLC assay method has been developed and validated for the determination of levetiracetam in bulk and pharmaceutical dosage form as per ICH guidelines [13].

MATERIALS AND METHODS

Levetiracetam standard was provided by Amoli organics Pvt Ltd (Baroda), India. "Levesam- 500 mg" tablets were procured from local market. Methanol, Acetone and all other reagents used in this study were of AR grade purchased from Merck Pvt. ltd, Mumbai.

Selection of analytical wavelength

The standard solution of Levetiracetam in methanol was scanned over wavelength range 200 to 400 nm by using UV-Visible spectrophotometer. Wavelength 210 nm was selected for analysis where Levetiracetam showed higher absorbance (Figure 2).

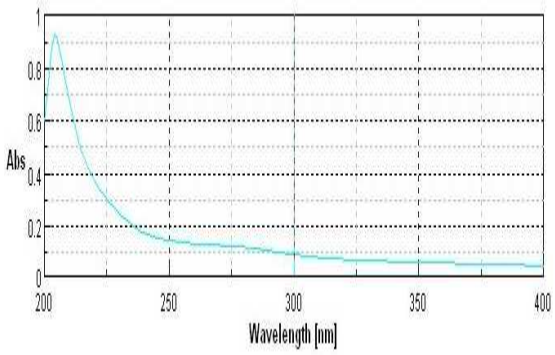


Fig. 2: UV spectra of Levetiracetam between 200 and 400 nm

Chromatographic conditions

Pre-coated silica gel 60 F_{254} TLC (E-Merck, Germany) plates (10x10 cm) were used as stationary phase. TLC plates were pre-washed with methanol and activated at 110°c for 10 min prior to application. The standard samples of levetiracetam were spotted on pre-coated TLC plates in the form of bands of length 4 mm using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat-5 applicator (Camag, Switzerland). The chromatographic development was carried using toluene: acetone: methanol (6:2:2 v/v) as mobile phase with chamber saturation time of 20 minutes and the migration distance of 80 mm. Densitometric scanning was performed using Camag TLC scanners 3 at 210 nm, operated by win CATS Software (Version 1.4.3, Camag). Deuterium lamp was used as a radiation source. All weighing was done on Shimadzu balance (Model AY-120).

Preparation of standard solution

A standard stock solution of levetiracetam was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. One ml of solution was further diluted to 10 ml to get 100 µg/ml solution of levetiracetam.

Validation

Linearity and Range

The calibration curve was obtained in the range of 500 - 3000 ng/band by applying different volumes (5-30 µl) of stock solution (100 µg/ml) on TLC plate. Each standard in six replicates was analyzed and peak areas were recorded. Standard calibration graph was plotted of peak area Vs concentration applied.

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra day studies, 3 replicates of 3 standard solutions (1000, 1500 and 2000 ng/band) were analyzed in a day and percentage RSD was calculated (Table 1). For the inter day variation studies, 3 standard solutions (1000, 1500 and 2000 ng/band) were analyzed on 3 consecutive days and percentage RSD was calculated (Table 2).

Accuracy

To check the accuracy of the method, recovery studies were carried out by over-spotting standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150 %. Basic concentration of sample chosen was 1000 ng/band. The areas were noted after development of plate. The drug concentration was calculated by using regression equation. (Table 3)

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for drug in sample was confirmed by comparing the Rf and spectra of the spot with that of standard drug spot. The specificity of the method was also ascertained by peak purity profiling studies by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on Win CATS software 5.

Robustness

The robustness of the method was studied, during method development, by small but deliberate variations in time from application to development (0, 30, 60, 120 min), and time from development to scanning (0, 30, 60, 120 min). One factor at a time was changed at a concentration level of 1000 ng/band to study the effect on the peak area of the drug.

Stress degradation studies

Stress degradation studies were carried under condition of acid/ base as well as neutral hydrolysis, oxidation, dry heat and photolysis. For each study, two samples were prepared. The blank subjected to stress in the same manner as the drug solution. Dry heat and photolytic degradation was carried out in solid state.

Table 1: Intraday study for LTC

Concentration(ng/band)	Intraday mean area*	% Recovery	SD	%RSD
1000	2711.5	96.39	17.06	0.62
1500	3719.8	99.63	19.01	0.51
2000	4608.1	98.64	12.19	0.26

* Average of 03 determinations

Table 2: Interday study for LTC

Concentration(ng/band)	Interday mean area*	% Recovery	SD	% RSD
1000	2762.4	99.16	28.21	1.03
1500	3669.9	98.72	32.59	0.87
2000	4696.3	101.70	45.67	0.98

* Average of 03 determinations

Table 3: Determination of accuracy for LTC:

Level	Conc.(ng/band)		Area	Average	% recovery	%RSD
50	1000	50	2542.6	2527.6	95.85	0.85
			2502.4			
			2536.5			
100	1000	1000	3174.3	3201.33	101.90	1.10
			3187.9			
			3241.5			
150	1000	1500	3402.2	3365.5	99.51	1.51
			3308.2			
			3389.1			

Degradation under alkali condition

1 ml working standard solution of LTC (1000 µg/ml) was mixed with 1 ml of 0.1 N NaOH (methanolic) and 8 ml of methanol. Solution was kept for overnight. 15 µl of the resulting solution was spotted on TLC plate. (Figure 4)

Degradation under acid condition

1 ml working standard solution of LTC (1000 µg/ml) was mixed with 1 ml of 0.1 N HCL (methanolic) and 8 ml of methanol. Solution was kept for overnight. 15 µl of the resulting solution was spotted on TLC plate. (Figure 5)

Degradation under neutral condition

1 ml working standard solution of LTC (1000 µg/ml) was mixed with 1 ml of distilled water and 8 ml of methanol. Solution was kept for overnight. 15 µl of the resulting solution was spotted on TLC plate. (Figure 6)

Degradation under oxidative condition:

1 ml working standard solution of LTC (1000 µg/ml) was mixed with 1 ml 3 % solution of H_2O_2 (methanolic) and 8 ml of methanol. Solution was kept for 2 hours. 15 µl of the resulting solution was spotted on TLC plate. (Figure 7)

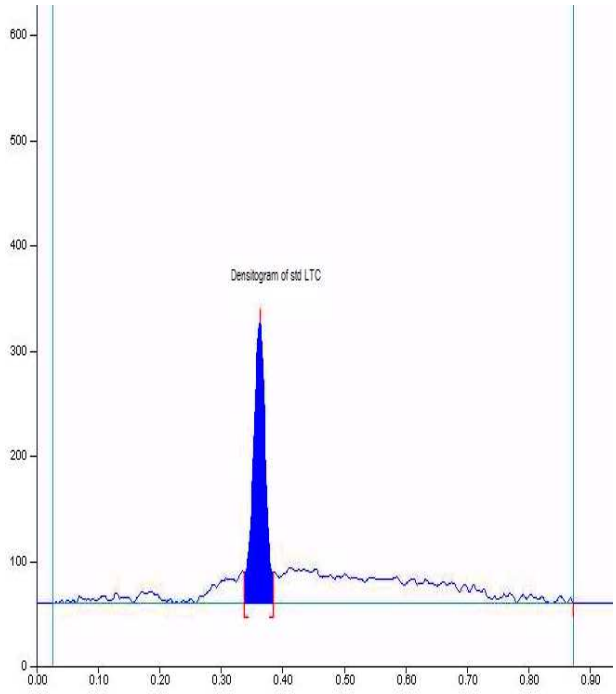


Fig. 3: Typical densitogram of standard Levetiracetam (1000 ng/band)

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (60^0 C) for a period of 24 hours. Sample was withdrawn, dissolved in methanol and diluted to get 1000 µg/ml. 1 ml was further diluted to get 100 µg/ml solution of which 15 µl volume was spotted on TLC plate. (Figure 8)

Photo-degradation studies

Photolytic studies were also carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool white fluorescent light to achieve an illumination of 1200 Lux.Hr. Sample was weighed, dissolved and diluted get 1000 µg/ml. 1 ml was further diluted to get 100 µg/ml solution of which 15 µl volume was spotted on TLC plate. (Figure 9) and (Figure 10)

RESULTS AND DISCUSSION

Optimization of mobile phase

Method development for levetiracetam (LTC) was started with the development of densitogram with neat solvents and combinations of Toluene, n-hexane, Ethyl acetate, and Methanol in different ratios. Toluene: Acetone: Methanol in the ratio of (6:2:2 v/v) was selected as the mobile phase for LTC, which resulted in good resolution and acceptable peak parameters. The Rf found to be 0.35 ± 0.005 for levetiracetam. (Figure 3)

The results were found to be linear in the concentration range 500 – 3000 ng/band with correlation coefficient of 0.997. The results of validation are summarized in Table 4.

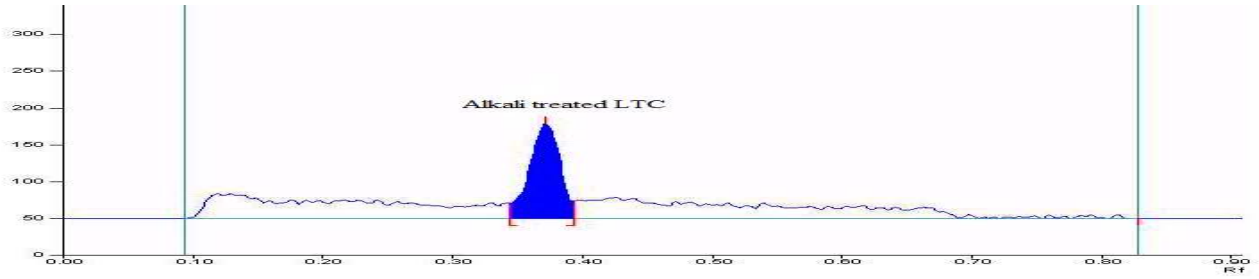
Table 4: Summary of validation study

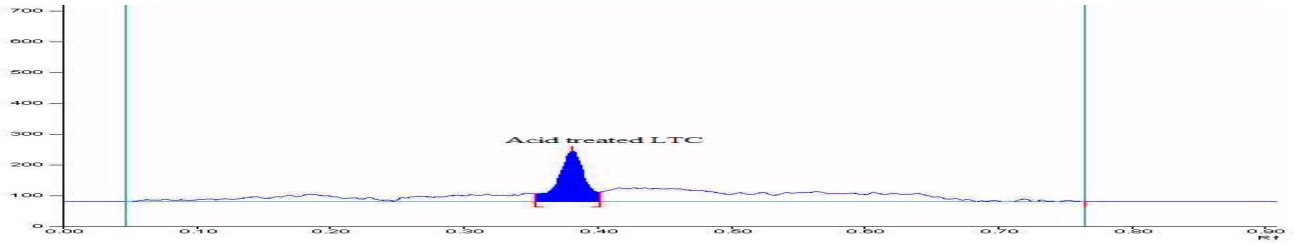
Sr. No.	Validation Parameter	Results
1.	Linearity	$y = 1.855x + 922.9$ ($R^2 = 0.997$)
2.	Range	500-3000ng/band
3.	Precision	%RSD
	A) Intraday precision	0.26 - 0.62
	B) Interday precision	0.87 – 1.03
4.	Accuracy	% recovery
	50%	95.85
	100%	101.90
	150%	99.51
5.	LOD	19.76
6.	LOQ	65.89
7.	Specificity	Specific
8.	Robustness	Robust

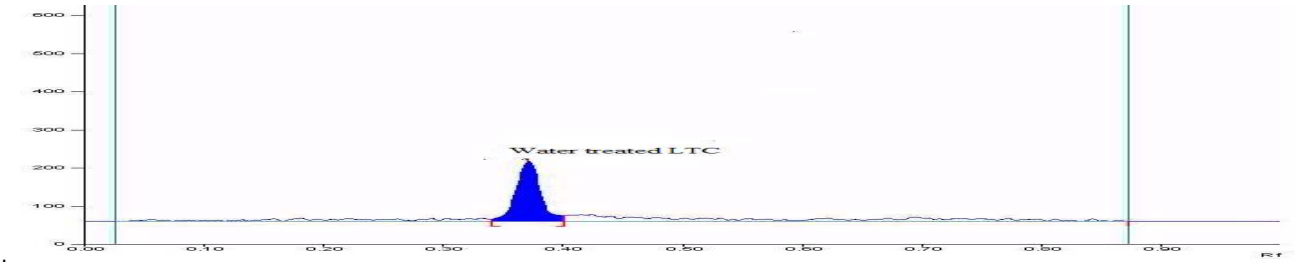
Table 5: Summary of stress degradation of Levetiracetam

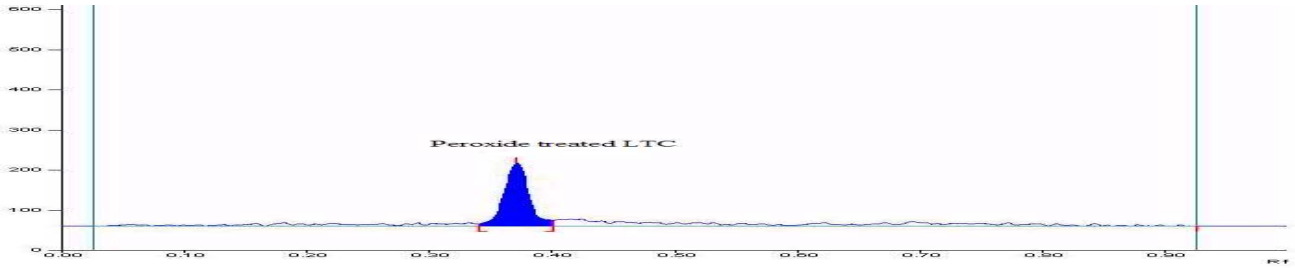
Stress Degradation Condition	Percent Assay	Percent degraded (%)	R_f of degradation product
Base (0.1 N NaOH, kept for overnight)	75.10	25.11	-
Acid (0.1 N HCl, kept for overnight)	73.38	26.61	-
H_2O_2 3% (kept for 2 hours)	93.50	6.5	-
Heat dry (80^0C, 24 Hours)	79.33	20.66	0.47
Photo stability (UV, 200 watt hrs/square meter Florescence, 1.2 million Lux. Hrs)	81.17	18.83	-
	75.22	24.78	

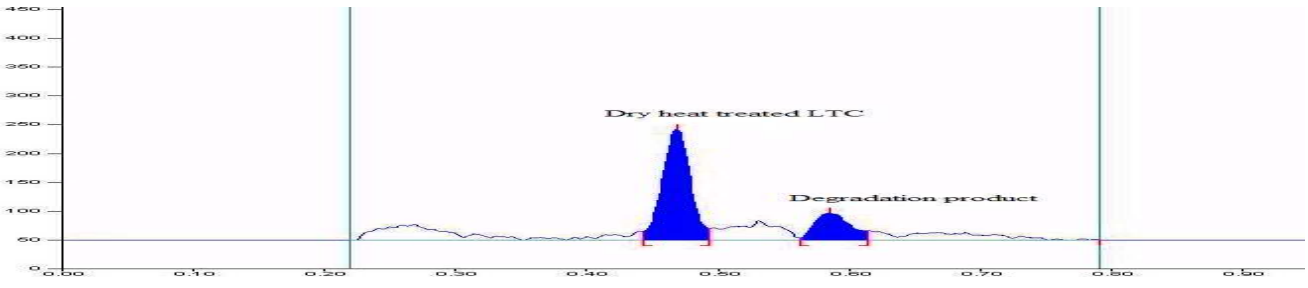
Drug was subjected to various forced degradation conditions. Chromatograph of LTC undr various stress conditions is shown in Figure 4. Although drug shown reduced percentage assay under all conditions; the peak for degradation product was only observed under dry heat exposure. Summary of stress degradation results is given in Table 5. Peak purity results greater than 0.999 indicate that peak is homogeneous in all stress conditions tested. The unaffected assay of drug in the tablet confirms the stability indicating power of the method.

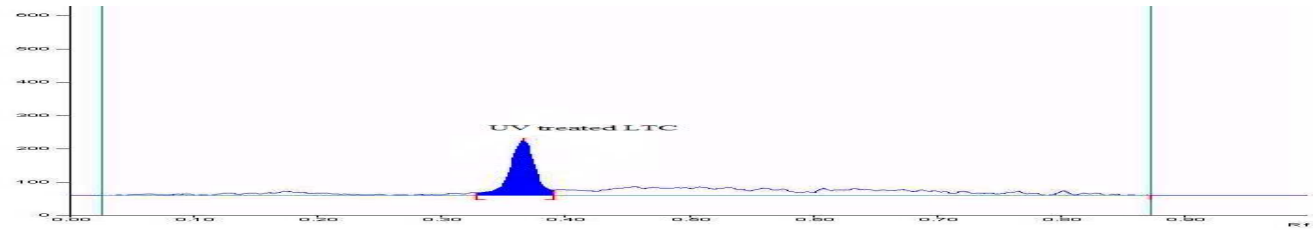

Alkali treated LTC


Acid treated LTC


Water treated LTC


Peroxide treated LTC


Dry heat treated LTC
Degradation product


UV treated LTC

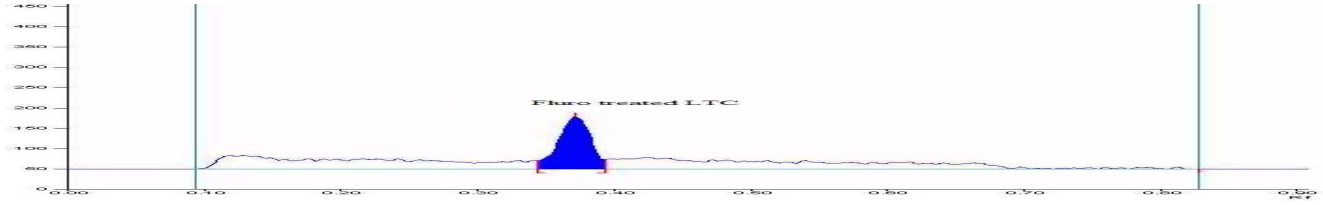


Fig. 4: Chromatogram of LCT I- Alkali treated, II- Acid treated, III- neutral degradation, IV- Oxidation, V- Dry heat, VI- photo degradation. (UV light) VII- photo degradation. (Fluorescence light)

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