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Research paper

Validated HPTLC method for the quantitation of levetiracetam in pure and tablet dosage form

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Abstract

A rapid, simple, precise and accurate high performance thin layer chromatographic method has been developed and validated for the estima-tion of Levetiracetam in bulk and tablet dosage form. The Levetiracetam was chromatographed on silica gel 60 F254 HPTLC plate as a sta-tionary phase. The mobile phase was Ethyl acetate: Methanol: Ammonia in the ratio of 7:1:2 respectively. It gave a dense and compact spot of Levetiracetam with an Rf value of 0.56. The quantitation was carried out at 210 nm. The method was validated in terms of linearity, accu-racy, precision and specificity. The statistical analysis proved that the developed method is accurate and reproducible with linearity in the range of 100 to 500 ng/spot. The limit of detection and limit of quantitation for Levetiracetam were 3.55 and 10.66 ng/spot respectively. The developed method can be employed for the routine analysis of Levetiracetam in the tablet dosage form.

Keywords: *Levetiracetam; HPTLC; Validation; ICH; Tablet Dosage Form.*

1. Introduction

Levetiracetam is an anticonvulsant drug used to treat the epilepsy [1]. Levetiracetam is a drug with in the pyrrolidine class that is used to treat various types of seizures [2]. Chemically it is known as pyrrolidinone and acetamide derivative. Levetiracetam may selectively prevent hyper synchronization of epileptic form burst firing and propagation of seizure activity. It is also used to treat neuropathic pain [3]. The chemical name of Levetiracetam is (S)-2-(2-oxopyrrolidin-1-yl) butanamide with molecular formula of $C_8H_{14}N_2O_2$ and a molecular weight of 170.20g/ml.

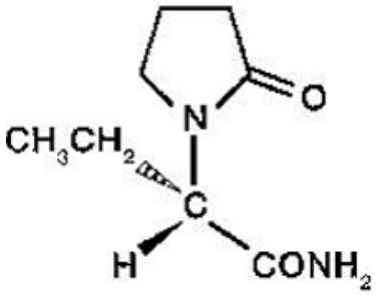

Fig. 1: Chemical Structure of Levetiracetam.

Literature survey revealed that there were few analytical methods have been reported for the determination of Levetiracetam in pure drug and pharmaceutical dosage forms by using UV spectrophotometric [4-7], HPLC [8-10] and HPTLC [11] so far.
The aim of present work is to develop and validate a novel, rapid, simple, precise and specific Zero order derivative UV Spectrophotometric method for estimation of Levetiracetam in bulk and tablet dosage form.

2. Experimental

2.1. Chemicals

Levetiracetam was obtained by Mylan pharma industry Pvt Ltd Bangalore, India. “Levipil- 500 mg” tablets were procured from local market. Methanol, Ethyl acetate and Ammonia obtained from the laboratory of Bharathi college of Pharmacy K M Doddi, Mandya.

2.2. Chromatographic condition

Pre-coated Silica gel $60F_{254}$ TLC plates (10×10 cm, layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) were purchased from Merck Ltd India, used as stationary phase. TLC plates were pre-washed with methanol and activated at 110ºc for 10min prior to use for chromatography. The standard sample of Levetiracetam were spotted at a distance of 1cm from the edge of the plate using 100µl sample syringe with a Camag Linomat-5 applicator. The plates were developed for 20 minutes in a camag chamber, previously saturated for 20 minutes with the mobile phase of Ethyl acetate: Methanol: Ammonia (7:1:2 v/v) and developed up to distance of 80mm. After development, plates were air dried and densitometric scanning was performed at a wavelength of 210 nm with camag scanner controlled by winCATS software.

2.3. Preparation of stock 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.

2.4. Method validation [12]

2.4.1. Linearity and range

The calibration curve was obtained in the range of 100 - 500 ng/spot by applying different volumes (1-5 µl) of stock solution (100 µg/ml) on TLC plate. Each standard of six determinations were analysed and peak areas were recorded. Standard calibration graph was plotted as peak area vs concentration used. The linearity of response for Levetiracetam was assessed in the concentration ranges 100-500 ng/spot, (Fig 2) the slope, intercept, and correlation coefficient were also determined. (Table 1).

2.4.2. Precision

The precision of the method was determined by intra-day and inter-day studies. In the intra-day studies was determined by analysing the range of 100-500 ng/spot in a day for three times and for inter-day studies was determined by analysing the range of 100-500 ng/spot on 3 consecutive days. The % RSD of the response such as peak area value were calculated and it was less than ±2. (Table 2).

2.4.3. Accuracy

To assess the accuracy of the developed method, recovery studies were carried out by spotting standard drug solution at three different levels 50%, 100% and 150 %. Basic concentration of sample chosen was 300 ng/band. The areas were noted after development of plate and shown good recovery studies. (Table 3).

2.4.4. Specificity

The specificity of the method was by analysing standard drug and sample. The specificity was confirmed by observing that the excipients present in the solution do not interfere in the response of analyte. In order to confirm the sensitivity limit of detection (LOD) and limit of quantification (LOQ) were calculated. (Table-4).

2.4.5. Ruggedness

The Ruggedness was determined by changing the analyst as analyst-1 and analyst-2 and observes the value %RSD it was less than ±2. (Table-4).

2.5. Analysis of the marketed formulation

Twenty tablets [Levipil, Sun pharma laboratories, Ltd.] were weighed and finely powdered. The powder equivalent to 10 mg of Levetiracetam was accurately weighed and transferred to 10 ml volumetric flask containing 5 ml methanol. The flask was shaken and volume was made up to the mark with methanol. The solution was filtered through Whatman filter paper (No. 41) to give a solution of concentration 1000 µg/ml. From the above solution pipette out 1 ml and make up the volume to 10 ml with methanol to give a solution containing 100 µg/ml. From this solution, appropriate volume was injected to the TLC plate. The analysis was carried out by two analysts.

3. Results and discussion

3.1. Optimization of the procedure

The pure drug was applied to the TLC plates and chromatographed with mobile phases Ethyl acetate: Methanol: Ammonia (7: 1: 2 v/v/v) was found to enable good resolution with a sharp and symmetrical peak of R_F 0.56. Well defined bands were obtained when the chamber was saturated with mobile phase for 20 min at room temperature.

3.2. Validation

3.2.1. Linearity

The calibration graph was linear over the range 100-500 ng per band. The regression data showed linearity was good over the concentration range investigated; this was apparent from the high value of the correlation coefficient. Typical linearity data are given in Table 1. The calibration curve (Fig. 2) and 3D view (Fig. 3) shows good correlation between Levetiracetam concentrations and peak areas (Fig. 4).

Table 1: Linearity Study of Levetiracetam

Sl no	Concentration (ng/spot)	Peak area ±Standard deviation*
1	0	0
2	100	828±10.8230
3	200	1631±5.8594
4	300	2409±3.7415
5	400	3143±9.5116
6	500	3902±14.5220

*Average of six determinations.

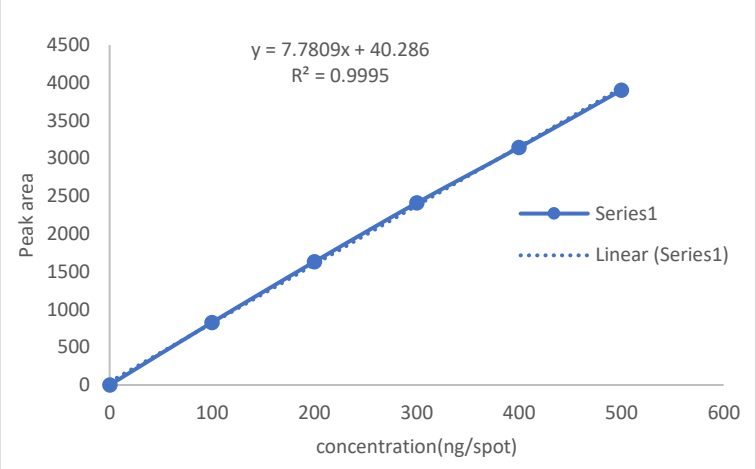

Fig. 2: Calibration Curve of Levetiracetam by HPTLC Method.

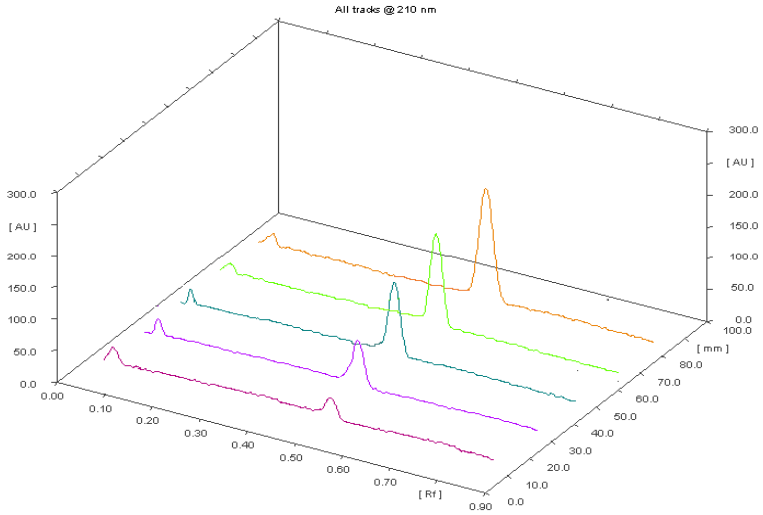

Fig. 3: 3D View of Levetiracetam by HPTLC Method.

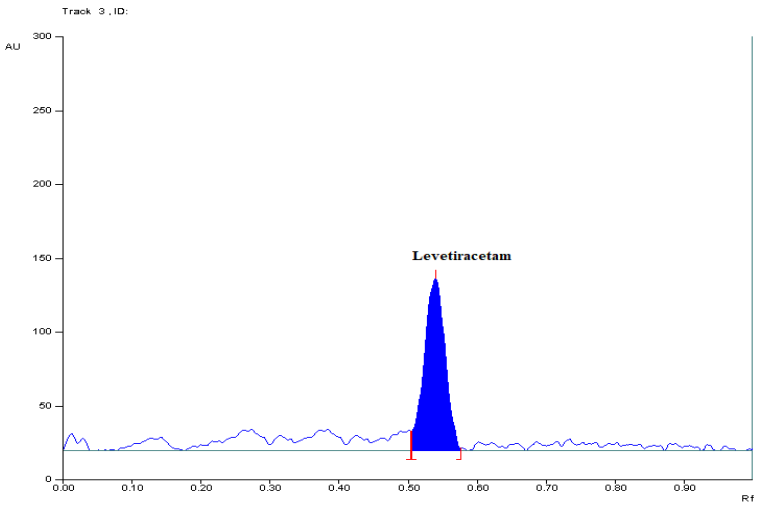

Fig. 4: Typical Densitogram of Levetiracetam.

3.2.2. Precision

Repeatability of sample application of peak area, as % R.S.D, was determined for concentrations 100-500 ng. % R.S.D for interday and intraday analysis was <2%. The values are shown in (Table 2).

Table 2: Precision Study of Levetiracetam

Concentration (ng/spot)	Intraday peak area ±Standard deviation*	%RSD	Interday peak area ±Standard deviation*	%RSD
100	837.6±4.9888	0.595	836.3±4.9216	0.588
200	1636.3±6.9442	0.424	1635.3±7.0395	0.430
300	2412±7.5865	0.314	2410.6±7.7602	0.322
400	3146.6±8.6538	0.275	3146.6±8.9566	0.284
500	3910.6±9.2855	0.237	3907.3±10.3387	0.264

*Average of three determinations.

3.2.3. Accuracy

When the method was used for subsequent analysis of Levetiracetam in Pharmaceutical dosage form spiked with 50, 100 and 150 % extra drug, recovery was 98-102 % of Levetiracetam as bulk and in dosage form (Table 3).

Table 3: Determination of Accuracy for Levetiracetam

Level	Concentration of sample (ng/spot)	Concentration of standard (ng/spot)	Average Peak area	%recovery ±Standard deviation*	%RSD
50	300	150	3154	99.94±0.3109	0.310
100	300	300	4678	99.83±0.2981	0.298
150	300	450	5862	100.33±0.1655	0.165

*Average of three determinations

3.2.4. Ruggedness

The Ruggedness was determined at concentration of 300 ng/spot by different analyst and values are shown (Table 4).

3.2.5. Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ for the proposed method were found to be 3.55 and 10.66 ng/spot, respectively.

Table 4: Method Validation Parameter

SL NO	Validation Parameter	Results
1	Linearity	Y=7.7809x+40.286 R^2 = 0.9995
2	Range	100-500ng/spot
3	Detection wavelength	210nm
4	R_f	0.56
5	LOD	3.5546ng/spot
6	LOQ	10.6647ng/spot
	Ruggedness	%RSD
7	Analyst-1	0.269
	Analyst-2	0.275

3.3. Analysis of marketed formulation

A single band at R_F 0.56 was observed in the densitogram of drug samples extracted from tablets. There was no interference from excipients commonly present in the tablets. The drug content was found to be 99.34% and 99.74%. The results are reported (Table 5).

Table 5: % Recovery of Formulation

Brand used	Standard drug Added	Theoretical content(ng/spot)	%Recovery*	%RSD
Levipil Tablet	0% level	100	99.34	0.37
	50%level	150	99.57	0.28
	100%level	200	99.42	0.40
	150%level	250	99.79	0.19

*Average of three determinations.

4. Conclusion

The developed HPTLC method combined with densitometric analysis was found suitable for determination of Levetiracetam. Statistical data analysis proves that the method is precise and reproducible for the analysis of Levetiracetam. The method was validated in accordance with ICH guidelines. The system being economical and can be employed for the routine estimation of the drug in tablet as well as in bulk drug analysis.

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