
Validated HPTLC Method for Estimation of Lamotrigine in Tablets

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A simple, specific and precise high performance thin-layer chromatography method has been developed for estimation of lamotrigine in its tablet dosage form. In this method, standard solution and sample solution of lamotrigine were applied on precoated silica gel G60 F₂₅₄ TLC plate and developed using a mixture of acetone:toluene:ammonia (70:30:5.0 v/v) as mobile phase. The quantification was done by densitometry at 312 nm. The method was quantitatively evaluated in terms of linearity, accuracy, precision, repeatability and specificity to prove its utility in the analysis of tablet dosage form.

Lamotrigine (3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine) is a new antiepileptic drug from triazine class of chemical compounds. It has recently been approved for the treatment of partial and generalized epileptic seizures as an add-on agent or as monotherapy and it is generally well tolerated'. It acts by blocking voltage dependent sodium channels in the neuronal membrane, thus reducing release of excitatory neurotransmitters, especially glutamate². Lamotrigine is not official in IP, BP or USP. HPLC and GC methods of analysis have been reported in literature for determination of lamotrigine in biological fluids³⁻⁵. No high performance thin layer chromatography (HPTLC) method has so far been reported for the estimation of lamotrigine in bulk and in pharmaceutical preparations. The present study describes the development and validation of a simple, specific, sensitive, accurate and precise HPTLC method for the determination of lamotrigine in tablet dosage form.

MATERIAL AND METHODS

Lamotrigine working standard was a generous gift sample from RPG Life Sciences Ltd., Mumbai. Silica Gel 60 F₂₅₄ TLC plates (20 X 20 cm, layer thickness 0.2 mm, E-Merck, Darmstadt, Germany) were used as the stationary phase. Twenty lamotrigine tablets (Lamotec-25, Cipla Ltd.,

Daman) were purchased from a local pharmacy. Toluene, acetone and ammonia of AR grade purity were procured from Merck Ltd., Mumbai.

Linomat IV sample applicator, twin trough developing chamber and TLC scanner III with CATS evaluation software (Version 4.06) were used in the studies (Camag, Muttenz, Switzerland).

Standard Preparation:

A sample of pure lamotrigine was accurately weighed (25 mg) and transferred in to a 25 ml volumetric flask. Lamotrigine was dissolved and diluted upto the mark with methanol to give a standard stock solution of 1 mg/ml. One milliliter of this stock solution was diluted further to 10 ml with methanol to give a working standard solution of 100 µg/ml.

HPTLC method and Chromatographic conditions:

The chromatographic estimation was performed using the following conditions, stationary phase was precoated silica gel 60 F₂₅₄ aluminum sheets (10x10 cm) and the mobile phase used was acetone:toluene:ammonia in the ratio of 7:3:0.5 v/v. The chamber saturation time employed was 15 min and the developing distance used was 7 cm. Scanning wavelength of 312 nm with a slit dimension of 3.0 x 0.45 mm and scanning speed of 10 mm/s were employed.

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Spotting parameters used were, 4 mm bandwidth, 3 mm space between two bands and a spraying rate of 10 s/ μ l.

Linearity of detector response:

Solutions of lamotrigine in the concentration range of 100-900 μ g/ml were prepared by diluting the standard stock solution of lamotrigine (1 mg/ml) with methanol. Of these, five microlitres were spotted as sharp bands on the precoated TLC plate. The plate was developed in the chamber, previously saturated for 15 min with mobile phase. The mobile phase was allowed to travel for 70 mm above the line of application of band. The plate was removed from the chamber and dried in hot air. Photometric measurements were performed at 312 nm in absorbance mode. Data peak area of each band was recorded.

Assay of tablets:

Twenty tablets were crushed and ground to fine powder. A weight equivalent to 25 mg of lamotrigine was transferred to a conical flask and extracted with methanol. The extract was filtered through Whatman filter paper No. 1 and the residue was washed with sufficient amount of methanol. The extract and the washings were pooled, transferred to a 250 ml volumetric flask and the final volume was made upto 250 ml with methanol to give a sample solution of 100 μ g/ml. A fixed volume of 4, 5 or 6 μ l of the working standard solution (100 μ g/ml) and 4 or 5 μ l of sample solution were spotted as sharp bands on the chromatoplate (multilevel calibration). After development, the bands of the drug were scanned at 312 nm.

Method validation:

The method was validated in terms of linearity, accuracy, inter-day and intra-day precision, reproducibility of measurement of peak area, reproducibility of sample application and specificity. The limit of quantification and limit of detection for lamotrigine were also determined. Accuracy of the analysis was evaluated by carrying out a recovery study. For that, known concentration of standard drug was added to a preanalysed tablet sample at three different levels namely 80, 100 and 120% and average recovery was calculated.

The intra-day precision was determined by analyzing standard lamotrigine solutions in the concentration range of 200 to 600 ng/spot for 3 times on the same day, while inter-day precision was determined by analyzing corresponding standards daily for a period of one week. Repeatability of measurement of peak area was determined by spotting 5 μ l

of lamotrigine standard solution on a TLC plate and developing the plate. The separated spot of lamotrigine was scanned 7 times without changing position of plates and relative standard deviation (RSD or % CV) for measurement of peak area was calculated. Repeatability of sample application was assessed by spotting 5 μ l of lamotrigine standard solution 7 times on a TLC plate by semiautomatic spotter, followed by development of plate and recording the peak areas for seven spots. The RSD for the peak area values was calculated.

The specificity of the proposed method was checked by spotting a sample solution of lamotrigine on the TLC plate and developing and scanning the plate as described earlier. Purity was also checked by overlaying the spectra of standard lamotrigine with the spectra of sample recorded on a TLC scanner in UV range.

RESULTS AND DISCUSSION

Literature survey indicated that various methods have been reported for analysis of lamotrigine in biological fluids. Most of them are either HPLC or GC methods, which are sophisticated, costly and time consuming. The above methods may turn out to be non cost effective for the routine analysis purposes such as assay and determination of content uniformity. Therefore, it was decided to develop a method of analysis of lamotrigine using HPTLC, a versatile speedy and cost effective technique.

Since lamotrigine is freely soluble in methanol, the tablet powder was extracted with methanol. Sonication for 10 min helped to extract lamotrigine completely from tablet matrix. Combination of toluene and acetone offered optimum migration ($R_f = 0.56 \pm 0.02$) and resolution of lamotrigine from other components of formulation matrix. On the other hand ammonia solution helped in making the peak sharper (fig. 1).

The amount of lamotrigine in tablet formulation was calculated on applying suitable dilution factor and comparing peak height and peak area of the standard and sample solutions. The assay of lamotrigine in tablet formulation, calculated as per peak area and peak height was found to be 99.9 ± 0.33 % and 100.6 ± 0.36 %, respectively (Table 1).

The linearity of response for lamotrigine was found in the range of 200 to 600 ng/spot with a correlation coefficient of 0.9943. The limit of detection and limit of quantification was found to be 3.6 and 10.9 μ g/ml, respectively. The percentage recovery was calculated using suitable diluting

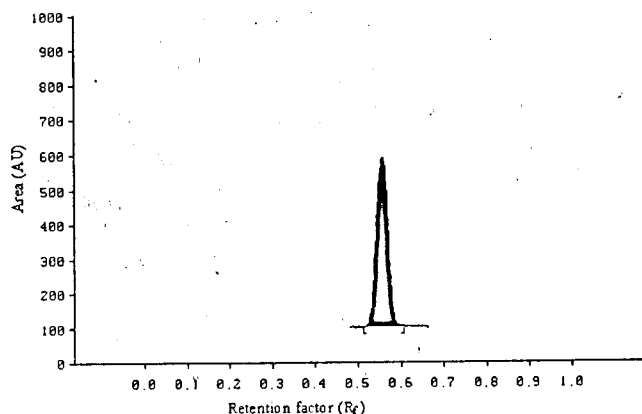


Fig. 1: Typical densitogram of lamotrigine from tablet.

factor. The average recovery value obtained was 100.6 % (Table 2).

The intra-day and inter-day coefficient was found to be in the range of 0.49 % to 1.5% and 0.25% to 2.75%, respectively. Lower values of intra-day and inter-day variation in the analysis indicate that the method is precise. The RSD for repeatability of measurement of peak area was found to be 0.86 % and RSD for measurement of peak areas for repeatability of sample application was found to be 0.81 %. Both the RSD values, for repeatability of measurement of peak area and sample application, were well below the instrumental specifications, ensuring proper functioning of HPTLC system.

It was observed that excipients present in formulation

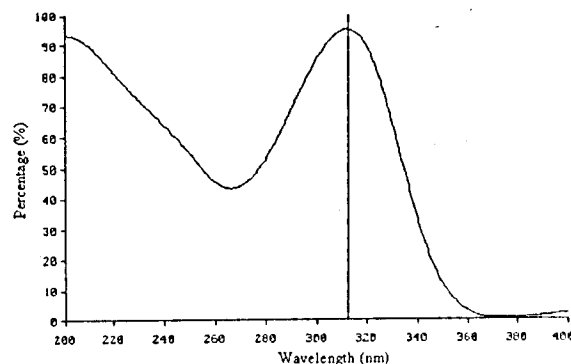


Fig. 2: Overlay absorption spectra.

Overlay absorption spectra of standard lamotrigine and lamotrigine from tablet, taken on the CAMAG TLC Scanner III.

did not interfere with peak of lamotrigine ($R_f = 0.56 \pm 0.02$). The purity was confirmed by overlaying the spectra of standard lamotrigine with the spectra of sample recorded on TLC scanner in UV range (fig. 2). The purity was also confirmed by comparing the spectra at three different levels i.e. at peak start (S), peak apex (M) and Peak end (E) and good correlation between these three spectra indicated the purity of lamotrigine peak and average purity was found to be 99.95 %. Different validation parameters⁶ for the proposed HPTLC method for determination for lamotrigine have been summarized in Table 3. The proposed HPTLC method was found to be rapid, simple, specific, sensitive, precise and accurate. Thus it can be employed for the routine quality control analysis of lamotrigine from tablets.

TABLE 1: ANALYSIS OF LAMOTRIGINE TABLET DOSAGE FORM

Labeled claim (mg)	Amount found \pm SD (mg)*		%Assay \pm SD*		%CV	
	Height	Area	Height	Area	Height	Area
25.0	25.1 \pm 0.09	25.0 \pm 0.08	100.6 \pm 0.36	99.9 \pm 0.33	0.4	0.3

*Average value \pm standard deviation of five determinations.

TABLE 2: ACCURACY OF HPTLC METHOD FOR LAMOTRIGINE

Amount added (mg)	Amount Recovered* (mg)	% Recovery	Average Recovery
20.0	19.9 \pm 0.6	99.5	100.6 %
25.0	25.5 \pm 0.3	102.0	
30.0	30.1 \pm 0.2	100.0	

*Average value \pm standard deviation of seven determinations.

TABLE 3: METHOD VALIDATION PARAMETERS

Parameter	Result
Linearity range (ng/spot)	200-600
Correlation coefficient (r)	0.9943
Limit of detection (LOD)	3.6 µg/ml
Limit of quantification (LOQ)	10.92 µg/ml
Accuracy	100.6 %
Precision (%CV)	
Repeatability of application (n=7)	0.8
Repeatability of measurement (n=7)	0.86
Inter-day (n=3)	0.25-2.75
Intra-day (n=3)	0.49-1.5
Specificity	Specific

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