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Validated Densitometric Method for the Quantification of Lamotrigine in Dosage Form

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Abstract: A simple, specific, accurate and precise high performance thin layer chromatography method has been developed for the estimation of Lamotrigine in tablet dosage forms. The quantification was carried out at 259 nm. Developed method was validated in terms of linearity, accuracy, precision, repeatability and specificity. Separation was achieved on silica gel 60 F_{254} plates with ethyl acetate: chloroform: water in the ratio of 9.0: 3.0: 2.5 v/v as mobile phase.The chamber saturation time employed was 15 min and the developing distance used was 4 cm. Densitometric quantification was performed at 240 nm by reflectance scanning.Limit of detection and limit of quantification of Lamotrigine were found to be 41 ng/spot and 122 ng/spot, respectively. The method was validated for precision, accuracy, specificity and ruggedness.
Key Words: Lamotrigine Validated TLC Densitometric.

Introduction

Lamotrigine[1] (The Merck Index, 2001), 3,5-diamino-6-(2,3-dichorophenyl)-1,2,4-triazine, [84057-84-1], $C_9H_7C_{l2}N_5$, mol. wt. 256.09. Lamotrigine [6-(2, 3-Dichlorophenyl)-1, 2, 4-triazine-3, 5-diamine] is a broad spectrum antiepileptic drug, chemically different from other anti-convulsants. [1-3] the mechanism of action of lamotrigine is inhibition of the release of excitatory neurotransmitters (aspartate and glutamate) and also involvement of the blocking of voltage dependent sodium channels. [4],HPTLC[5] .Lamotrigine is effective for treatment of partial and generalized tonic, clonic seizures as a single drug or as an adjuvant with other anti epileptic drugs.[6]We are discusses The aim of the present study was to new method develop and validate a simple, for the lamotrigine. The primary goal was to develop and validate a HPTLC method for the rapid quantization of the drug. Author of the article and his research team has developed a HPTLC Method development different pharmaceutical dosage form[7-11].In this manuscript we report a simple, rapid, precise and accurate HPTLC method for simultaneous analysis of in Lamotrigine pharmaceutical preparations

Materials and methods

All the chemicals used in the experiment were of analytical grade. Lamotrigine tablet was procured as local market sample from Torrent Pharma Ltd., India.

Equipment:
The chromatography estimation was performed using the following conditions: stationary phase was precoated silica gel 60 F_{254} aluminum sheets (10 x 10 cm) and the mobile phase used was ethyl acetate : chloroform : water in the ratio of 9.0 : 3.0 : 2.5 v/v . The chamber saturation time employed was 15 min and the developing distance used was 4 cm. Scanning wavelength for lamotrigine was 259 nm with slit dimensions of 5.0 x 0.45 mm and scanning speed of 10 mm/s were employed. UV absorption spectrum of lamotrigine is shown in Figure1.Spotting parameters used were, 5 mm bandwidth, 10 mm space between

two bands and spraying rate 10 s/µl. The optimized chamber saturation time for mobile phase was 30 min at room temperature (37^0C) at relative humidity. The length of chromatogram run was 8 cm. The average development time was 30 min. After development the plate was dried in an oven at 80°C for 15 min. Densitometric scanning at λ = 240 nm, using a deuterium light source, was then performed with a Camag TLC Scanner equipped with win CATS Software Version 1.3.4. The slit dimensions were 5.00 mm × 0.45 mm and 10 mm/s scanning speed was employed.The length of chromatogram run was approximately 4 cm. Subsequent to the development; RP- TLC plates were dried in the current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner three in reflectance absorbance mode at 240 nm. The source of radiation utilized was deuterium lamp emitting continuous UV spectrum between 200 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of diffused light.

HPTLC method and chromatographic conditions

Preparation of standard solution:

Lamotrigine (100 mg) was accurately weighed and transferred to 25 ml volumetric flask and volume was made up to the mark with methanol to give a standard stock solution of 10.0 mg/ml. The aliquots of stock solution were transferred to 10 ml volumetric flasks and the volume of each was adjusted to 10 ml with methanol to obtain working standard solution containing 10, 25, 35, 45, 60 and 80 µg/ml of Lamotrigine respectively.

Calibration curve for Lamotrigine:

Standard solutions of Lamotrigine (10 µl) were applied in triplicate on TLC plate. The plate was developed in a solvent system of ethyl acetate: acetic acid: water in the ratio of 7.5:1.5:1 v/v up to distance of 7cm. After development, the plates were dried in hot air and scanned at 296 nm. The peak areas were recorded. Calibration curve of Lamotrigine was obtained by plotting peak area *vs* concentration of Lamotrigine applied.

Assay of tablets

Twenty tablets of Lamotrigine were crushed and ground to fine powder. A powder equivalent to 100 mg of drug was transferred to a conical flask and extracted with ether (4 X 25 ml) by sonication. The extracts were filtered through Whatmann No. 1 filter paper and the residue was washed with sufficient amount of methanol. The extract and its washings were pooled, transferred to a 100 ml volumetric flask and the final volume was made up to 100 ml with methanol to give a sample solution of 100 µg/ml. A fixed volume of working standard solutions (65 µg/ml) and sample solutions were spotted as sharp bands on the TLC plate and the plate was developed as mentioned above. The band of the drug was scanned at 259 nm.

Method validation:

Validation of the developed HPTLC method was carried out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1)[12-14] for Linearity, Accuracy, Precision, Limit of Detection, Limit of Quantification, Repeatability, Specificity and Robustness.

Linearity and calibration curve

Linearity of the method was evaluated by constructing calibration curves at eight concentration levels. Aliquots of standard working solution of Lamotrigine were applied to the plate to obtain concentration in the range of 200 to 600ng/spot. The calibration curves were developed by plotting peak areas Vs Concentrations with the help of Win-CATS software. Chromatogram was developed in a twin trough glass chamber; using 15 minutes chamber saturation time. The length of chromatogram run was 55 mm. The developed plates were air-dried. Scanning was performed in UV mode at 299nm.

Precision:

To evaluate intra-day precision, three samples at three different concentrations (6µl) were analyzed on the same day. The inter-day precision was studied by comparing assays performed on three different days. The precision of an analytical method expresses.

Repeatability:

Repeatability of measurement of peak area was determined by spotting 8 µL of standard drug solution on TLC plate. After developing the plate separated spot of Lamotrigine was scanned six times without changing the position of the plate.

Accuracy:

Recovery studies of the drugs were carried out for determining accuracy parameter. It was done by mixing known quantity of standard drug with the analyzed sample formulation and the contents were reanalyzed by the proposed method. Recovery studies carried out at 100 and 50% levels. The percentage recovery and its %RSD were calculated.

Specificity:

The specificity of the proposed method, Lamotrigine was spotted on TLC plate, developed and scanned as described earlier. The UV spectrum of standard Lamotrigine was also compared with spectrum of Lamotrigine extracted from tablet. The peak purity of

Lamotrigine was assessed by comparing their respective spectra at peak start, peak apex and peak end positions of the spot.

Robustness:
The parameters selected for the robustness study were mobile phase composition, chamber saturation time and solvent migration distance. By introducing small changes in these parameters the effects on the results were examined.

Limit of Detection and Limit of Quantification
The limit of detection (LOD) and limit of quantitation (LOQ) was determined on the basis of signal to noise ratio. LOD was the amount of the applied sample producing a peak area that is equal to the sum of the mean blank area and three times the standard deviation. LOQ was the amount of the applied sample producing a peak area that is equal to the sum of the mean blank area and ten times its standard deviation. Stock solution of Riluzole (0.1 mg mL^{-1}) was prepared and different volume of stock solution in the range 200 to 600 ng were spotted in triplicate. The amount Riluzole by spot versus average response (peak area) was graphed and the equation for this was determined. The standard deviations (S.D.) of responses were calculated. The average of standard deviations was calculated (A.S.D.). Detection limit was calculated by (3.3 × A.S.D.) / b and quantification limit was calculated by (10×A.S.D.) / b, where "b" corresponds to the slope obtained in the linearity study of method.

Table 1: Analysis of Lamotrigine by proposed method

Sample	Label claim (mg/tablet)	Amount ± SD found (mg/tablet)[a]	% Assay ± SD[a]	Amount Added (mg)	Recovery (%)	Average Recovery
1	100	100.06 ± 0.84	99.96 ± 0.04	10.0	99.97	100.21%
				15.0	100.11	
				20.0	99.89	

[a]Mean ± standard deviation of five determinations. [b]Recovery study was performed on one formulation only.

Table 2: Method Validation Parameters

PARAMETERS	RESULTS
Linearity range	98 – 590 ng/spot
Correlation coefficient	0.9990
Limit of detection (LOD)	41 ng/spot
Limit of quantification (LOQ)	122 ng/spot
Accuracy	100.21%
Precision (%CV)	
Repeatability of application (n=7)	0.49
Repeatability of measurement (n=7)	0.26
Inter day (n=3)	0.20-1.03
Intraday (n=3)	0.85-2.18
Specificity	Specific

Table 3-Recovery Studies of Lamotrigine

Lamotrigine			
Label claimed	**Excess drug added to the analyte (%)**	**Amount recovered(ng)**	**% recovery**
100	0	99.96	99.92
	80	100.03	100.04
	100	101.09	101.11
	120	101.43	101.02

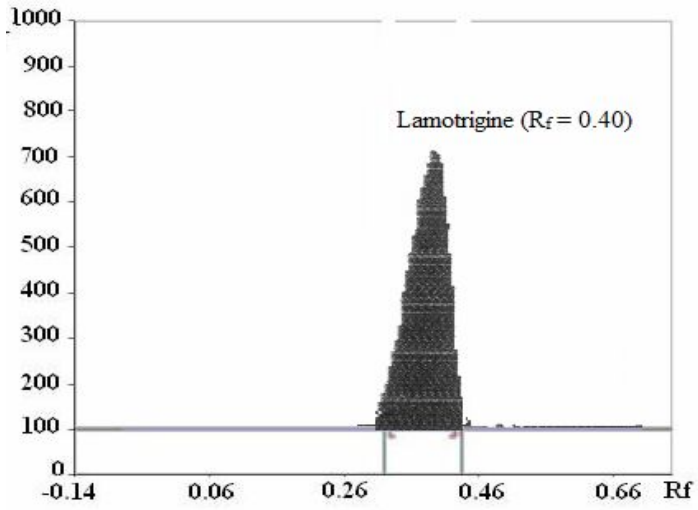

Fig. Chromatogram of Lamotrigine (R_f = 0.40)

Results and Discussions

A representative calibration curve obtained by plotting peak area of compound against the concentration over the range of 100 to 600 ng/spot. The slope, intercept and correlation co-efficient values were found to be 10.443 and 0.9990 respectively.Of the various mobile phases tried, the one containing ethyl acetate: acetic acid: water in the ratio of 7.5:1.5:1 was found to be suitable for Lamotrigine (R_f = 0.40). Limit of detection and limit of quantification of Lamotrigine was found to be 41 ng/spot and 122 ng/spot, respectively. Estimation was possible by using the same concentration of sample solution (500 ng/spot). The identity of the Lamotrigine in the sample extracts was confirmed by overlaying the UV absorption spectra with that of the reference standard using CAMAG TLC Scanner III. The average of percent recoveries at three different levels was found to be 99.98%. The content of Lamotrigine estimated in the sample extracts of dosage forms, by the proposed method was found to be in 100.09 % and 100.73 %, respectively.The RSD for measurement of peak area was calculated and was found to be 0.88%. In repeatability of sample application the %RSD for the peak area values were calculated and found to be 1.06 %. The RSD values for measurement of peak area and sample application were both below the instrumental specifications (i.e.1%); ensuring proper functioning of HPTLC system. The % recovery of Lamotrigine was found to be 99.84; 100.11, which is satisfactory. Robustness tests examine the effect of the operational parameters on the analysis results. By introducing small changes in mobile phase composition. The deviation obtained by deliberate changes in the aforementioned parameters was below 2% RSD which conforms the robustness of the method.

Conclusion:

The developed HPTLC method for the determination of Lamotrigine is simple, precise, specific, accurate, selective, sensitive and reproducible. The amounts found in formulations well agreed with label claim.

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