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Development and Validation of HPTLC Method for Estimation of Lacosamide in Bulk Drug and in Tablet Dosage Form

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1. Abstract: A new, economic, precise and rapid high-performance thin-layer chromatographic (HPTLC) method was developed and validated for quantitative determination of Lacosamide. The HPTLC separation was achieved on an aluminium-backed layer of silica gel $60F_{254}$ using toluene: methanol (7.5ml:2.5ml, v/v) as mobile phase. Quantitation was achieved by densitometric analysis at 258 nm over the concentration range of 2000-12000 ng/spot. The method was found to give compact spot for the drug (R_f 0.55). The linear regression analysis data for the calibration plots showed good linear relationship with r^2 = 0.9972. The method was validated for precision, recovery, repeatability, and robustness as per the International Conference on Harmonization guidelines. The minimum detectable amount was found to be 364.88 ng/spot, whereas the limit of quantitation was found to be 1105.72 ng/spot. Statistical analysis of the data showed that the method is precise, accurate, reproducible, sensitive and selective for the analysis of Lacosamide. The method was successfully employed for the estimation of Lacosamide as a bulk drug and in Tablet dosage form.
Key words: Lacosamide, HPTLC, Quantitative analysis.

2. INTRODUCTION:

Lacosamide[1](Figure-1) is a member of a series of functionalized amino acids and not official in any pharmacopoeia. It selectively enhances slow inactivation of voltage-gated sodium channels and modulates collapsin response mediator protein-2 (CRMP-2) involved in neuronal differentiation and control of axon outgrowth [1]. It has one chiral centre and is administered as R-form[1]. Lacosamide is chemically (R)-2-acetamido-N-benzyl-3methoxy propionamide used to treat partial-onset seizures in people with epilepsy who are at least 17 years old[1]. The literature survey revealed that there were some analytical methods reported for lacosamide like HPLC[2-4]. The review prompted us to develop a simple, precise and economic HPTLC method for the estimation of Lacosamide in bulk and in tablet dosage form.

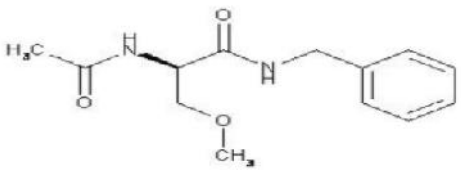

FIGURE:- 1 STRUCTURE OF LACOSAMIDE

3. MATERIALS AND METHODS:

3.1. Apparatus and reagents

The HPTLC system (Camag, Muttenz, Switzerland) consisted of Limomat V autosprayer connected to a nitrogen cylinder, a twin trough chamber (20 × 10 cm), a derivatization chamber, and a plate heater. Precoated silica gel 60 F_{254} TLC plates (10 × 10 cm, layer thickness 0.2 mm (E. Merck KGaA, Darmstadt, Germany) was used as stationary phase. TLC plates were prewashed twice with 10 mL of methanol and activated at 80^0C for 5 min prior to sample application. Densitometric analysis was carried out using a TLC scanner III with winCATS software. All solvents are of AR Grade and gratis sample of Lacosamide was collected from Torrent Pharmaceuticals Ltd. and tablet samples were obtained from local market.

3.2 HPTLC Method and Chromatographic Conditions

3.2.1. Sample Application

The standard and formulation samples of Lacosamide were spotted on Precoated TLC plates in the form of narrow bands of lengths 6 mm. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nL/s.

3.2.2. Densitometric Analysis and Quantitation Procedure

Densitometric scanning was performed on Camag TLC scanner III in absorbance mode and operated by winCATS planar chromatography version 1.3.4. The source of radiation utilized was deuterium lamp. The spots were analyzed at a wavelength of 258 nm. The slit dimensions used in the analysis were length and width of 5 mm and 0.45 mm, respectively, with a scanning rate of 20 mm/s. These are selected as recommended by the CAMAG TLC Scanner III manual.

3.2.3. Preparation of Lacosamide Standard Stock Solution

Stock solution was prepared by weighing lacosamide (50 mg). Weighed powder was accurately transferred to a volumetric flask of 50 mL and dissolved in and diluted to the mark with methanol to obtain a standard stock of solution of lacosamide (1000 µg/Ml).

3.2.4. Linearity and calibration curve

Linearity of the method was evaluated by constructing calibration curves at six concentration levels. Calibration curves were plotted over a concentration range of 2000–12000 ng/spot. Aliquots of standard working standard working solution of Lacosamide were applied to the plate(2,4,6,8, and 10 µL/spot).Calibration curves were developed by plotting peak area versus concentrations (n=6) with the help of the winCATS software.

3.2.5. Validation of developed method

Validation of the developed HPTLC method was carried out as per the International Conference on Harmonization (ICH) guidelines Q2B for specificity, sensitivity, accuracy, precision, repeatability, and robustness [5].

4. RESULT AND DISCUSSION:

To develop HPTLC method of analysis for Lacosamide for routine analysis, selection of mobile phase was carried out on the basis of polarity. A solvent system that would give dense and compact spots with appropriate and significantly different *Rf* value for Lacosamide was desired.Various solvent systems such as methanol:chloroform, ethyl acetate:methanol, hexane:methanol, toluene: methanol were evaluated in different proportions. Among these, the solvent system comprising of toluene:methanol (7.5:2.5,v/v) gave good separation of Lacosamide from its matrix with accuracy, specificity intraday and interday precision, repeatability of measurement of peak area as well as repeatability of sample application . The method was found to be linear in the range of 2000-12000ng/spot, $y = 0.1363x + 251.19$, $r^2 = 0.9972$ in four replicates. The signal to noise ratios of 3 and 10 were considered as LOD and LOQ respectively. The intraday precision was determined by analyzing standard of drug solution in the concentration range of 6000 ng/spot and 10000 ng/spot for three times on same day while interday precision was determined by analyzing corresponding standards daily for three days over a period of one week. To confirm the specificity of proposed method the solution of formulation(tablet) was spotted on TLC plate which was then developed and scanned. It was observed that the excipients present in the formulation did not interfere with peak of Lacosamide. Recovery studies of the drug were carried out for the accuracy parameters. These studies carried out at three different levels namely 80, 100 and 120%. The result of recovery study indicates the proposed method is accurate for estimation of Lacosamide in Tablet dosage form.

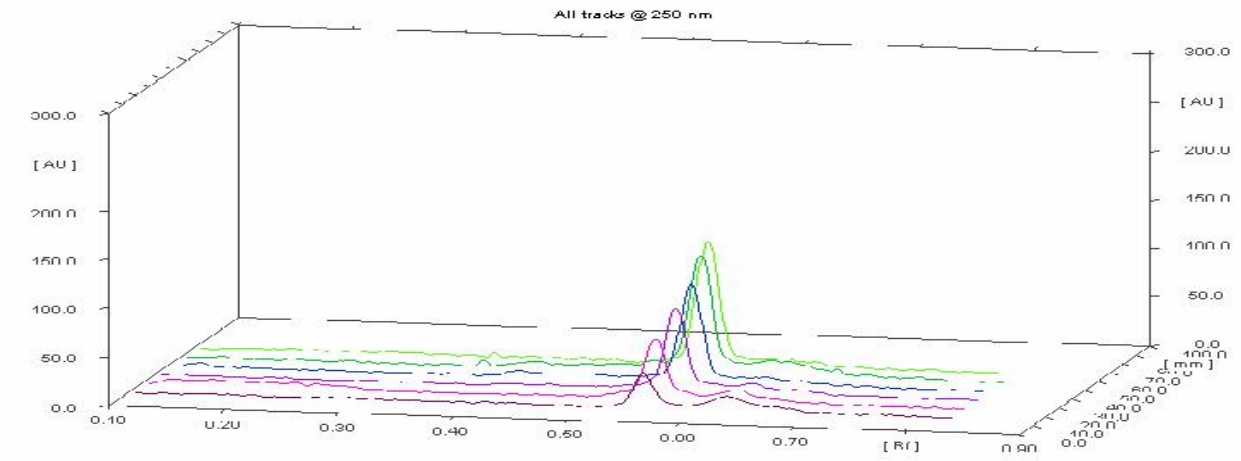

FIGURE 2:- 3D REPRESENTATION OF DENSITOGRAM FOR CALIBRATION CURVE OF LACOSAMIDE

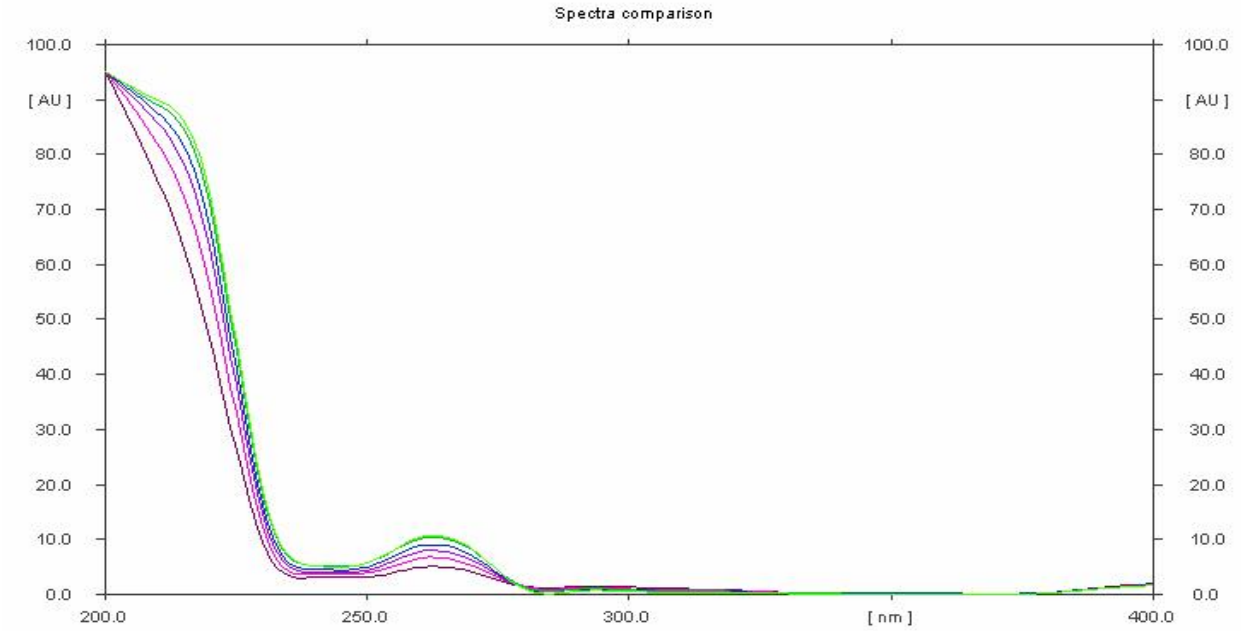

FIGURE 3:- UV ABSORPTION (REFLECTANCE MODE) OF THE CORRESPONDING SPOTS FOR LACOSAMIDE

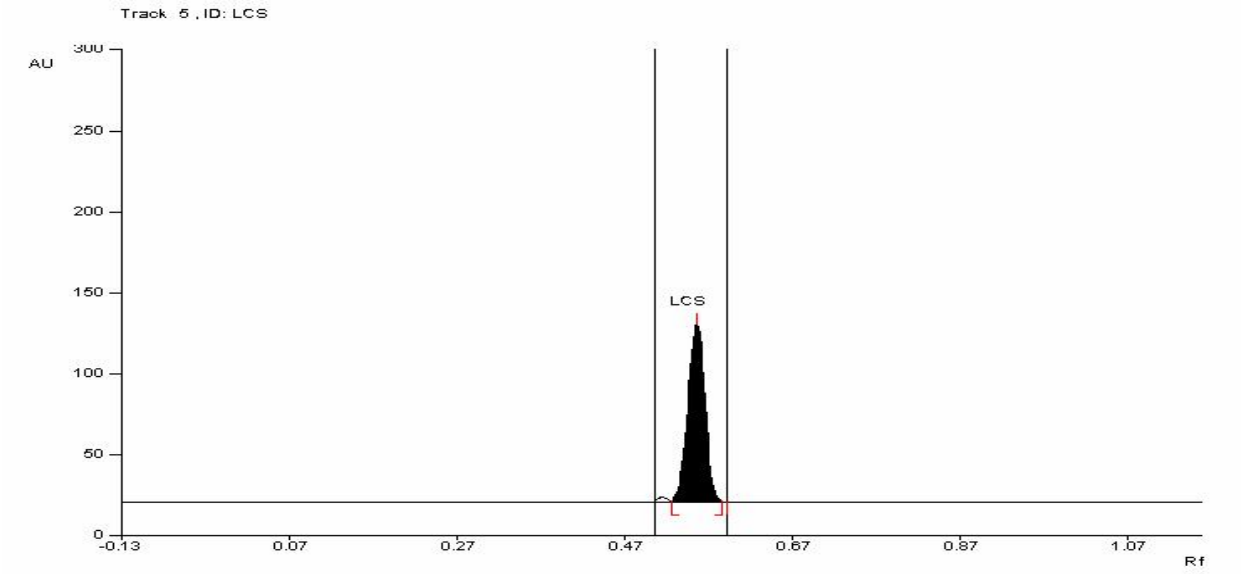

FIGURE 4:- HPTLC CHROMATOGRAM OF LACOSAMIDE STANDARD SOLUTION

TABLE 1. RESULT OF CALIBRATION READING FOR LACOSAMIDE

CONCENTRATION (ng/spot)	AREA(MEAN+STADARD DEVIATION)n=4	%RSD
2000	490.725±1.532	0.3123
4000	840.425±8.875	1.0560
6000	1086.2±21.406	1.9707
8000	1352.875±24.665	1.8231
10000	1642.325±24.867	1.5138
12000	1859.3±9.081	0.4884

TABLE 2. STATISTICAL DATA OF LACOSAMIDE

PARAMETERS	RESULT
LINEAR RANGE(ng/spot)	2000-12000
SLOPE	0.1363
INTERCEPT	251.19
STDEVIATION OF SLOPE	0.000519
STDEVIATION OF INTERCEPT	7.1829
LOD(ng/spot)	364.88
LOQ(ng/spot)	1105.72
CO-RELATION CO-EFFICIENT	0.9972

TABLE 3. ASSAY RESULT OF MARKETED FORMULATION

FORMULATION	ACTUAL CONCENTRATION	%PURITY	LIMIT
TABLET	6000ng/spot	99.78	99%-101%

TABLE 4. VALIDATION PARAMETER

SUMMARY OF VALIDATION PARAMETER	
Recovery(%)	99.67-100.18
Repeatability(%RSD)	1.1941
Precision(%RSD) Intra-day(n=3) Inter-day(n=3)	 1.14-1.62 0.95-1.42
Specificity	Specific
Selectivity	Selective

5. CONCLUSION

The proposed method is simple, sensitive, accurate, precise, reproducible, an applicable for the routine estimation of Lacosamide in bulk and its pharmaceutical dosage forms.

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