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A REVIEW ON HPTLC METHOD DEVELOPMENT AND VALIDATION FOR LACOSAMIDE IN TABLET AND BULK FORMULATIONS

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Abstract:

The objective of present work of review article was to study different HPTLC method developments for Lacosamide. In this review article we are proposed to add some points regarding method development and validation parameter, beneficial to quality assurance to determine the drug concentrations. A simple, selective, precise, and reproducible high-performance thin-layer chromatographic (HPTLC) method for the analysis of Lacosamide in bulk drug and tablet dosage form was developed. Statistical analysis of the obtained data showed the selectivity of the proposed method for Lacosamide estimation as a bulk drug and tablet dosage form. The present investigation demonstrated that the developed methods were successfully applied for pre- clinical and clinical studies.

Keywords: HPTLC, Lacosamide, method development, Validation.

Introduction:

Lacosamide (shown in below) is chemically known as (2R)-N-benzyl-2-acetamido-3- methoxy propanamide. Its chemical formula is C13H18N2O3 with a molecular weight 250.294 g mol-1. It's a white to light yellow powder that disperses just little in water, acetonitrile, and ethanol. The anticonvulsant drug lacosamide is used to treat partial-onset seizures. [1-2].

Fig. Structure of Lacosamide

Lacosamide is a functionalized amino acid that has activity in the maximal electroshock seizure test and is used in association to other medications to treat partial-onset seizures and diabetic neuropathic pain. According to recent studies, unlike other antiepileptic drugs like carbamazepine or lamotrigine, which slow the recovery from inactivation and reduce the ability of neurons to fire action potentials, Lacosamide only affects neurons that are depolarized or active for long periods of time, which are typical of neurons at the epileptic seizure's focal point. [3-4]

A review of the literature indicates a variety of HPLC tools for assessing the S (-) enantiomer of Lacosamide in bulk and pharmaceutical solutions [5], RP–HPLC [6-7], HPLC-UV in human plasma [8], Lacosamide spectrophotometric estimation in bulk and tablet dose forms has been confirmed[9], There are reports on the development and validation of UV spectroscopy methods for estimating Lacosamide in bulk and formulations [10-11] and HPTLC methods for estimating Lacosamide in bulk drug and tablet dosage form[12].

Our goal is to develop an HPTLC tool for estimating Lacosamide in bulk and tablet dosage forms that was approved [13] and had a lower limit of detection and quantification than Kamdar et al. (2012) [12].

I. Material Methods:

1.1. HPTLC Method:

A new, cost-effective, precise, and fast high-performance thin-layer chromatographic (HPTLC) method was developed and validated for the quantitative detection of Lacosamide. Over an aluminium-backed layer of silica gel 60F254, the HPTLC separation was achieved using toluene: methanol (7.5ml: 2.5ml, v/v) as the mobile phase. The concentration range of 2000-12000 ng/spot was evaluated by densitometric analysis at 258 nm. The method was discovered in order to offer a little place for the drug (Rf 0.55). The linear regression analysis data from the calibration plots revealed a satisfactory linear relationship with r2 = 0.9972. The quantitation limit was determined to be 1105.72 ng/spot, with lowest detectable quantity was 364.88 ng/spot.

1.1.1.Chloroform, methanol, formic acid, acetic acid, ammonia, and water have been used in various combinations for first separation. The mobile phases used were methanol: ammonia (6:4% v/v) and chloroform: water (4:6). Acetonitrile: water (4:6 % v/v) and Methanol: water (5:5 % v/v) were also tested.

Methanol: Ammonia (6:4 % v/v) was used to separate the substance, and the Rf value was found to be 0.90. The Rf value of Lacosamide was higher than expected (ranged from 0.2 to 0.8). (P.D. Sethi and colleagues) As a result, the same mobile phase is being used to test several ratios. The approach was chosen because the drug was extracted with good resolution in an 8:2 % v/v ratio of acetonitrile to water. The detection wavelength for the study was chosen to be 257 nm. Compares the spectral alignment of the standard Lacosamide with the sample solution Lacosamide in fig 1.

In methanol, a stock solution of Lacosamide at 1 mg/mL was produced. In the Twin Trough Chamber, chromatograms of Lacosamide were generated from a stock solution concentration range of 1g to 6g/ spot. Figures 2-7 shows the linearity chromatograms. When the calibration graph with concentration vs peak area was plotted, the correlation coefficient for Lacosamide was found to be 0.9992. Figure 8 shows the calibration graph for Lacosamide. Table no.1 shows optical properties like LOD, LOQ, Slope, Intercept, Regression equation, and correlation coefficient.

The approach was used to analysis the formulation. Lacosamide was discovered to have a purity of 99.13 percent 1.1737. Lacosamide 1.1840 % RSD values. The method's precision is validated by a six-time repeatable analysis of 99 formulations. Figures 9 through 14 show the chromatograms. Table no. 2 summarises the data.

Intraday and Interday analysis validated the method's intermediate precision. The formulation analysis was carried out three times on the same day and once on each of the three successive days. Lacosamide has a % RSD value of 0.3502 and 1.4218 for intraday and interday analyses, respectively. Tables 3and 4shows the results of the analyses.

The results showed that the method's precision had been further confirmed. The recovery analysis confirms the method's accuracy. The standard drugs were added to the pre-analyzed formulation in three different concentrations. Lacosamide was found in amounts ranging from 99.09 % to 102.36 %. 1.6790 was found to be the % RSD value. The low % RSD values show that the excipients did not interfere with the analysis. Figures 15-17 show the peaks of the produced chromatograms. Table no.5 shows the results of the recovery analysis.

1.2. Chemicals and reagent:

Glenmark Pharmaceuticals Ltd., Mumbai, Maharashtra, India, provided a free supply of lacosamide pure drug. All of the analytical solvents and reagents were grade from Merck in Mumbai, India.

1.3. HPTLC instrumentation:

The integrated software Win-Cats (V 3.15, Camag) was used for analysis. The standard and sample solutions were spotted in the form of 6 mm wide bands with a Camag 100 l sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel pre-coated aluminium plate 60F-254 plates (20 x 10 cm) with 250 m thickness (E. Merck, Darmstadt, Germany), supplied by Anchrom technologist, Mumbai. The bands were applied at a steady rate of 150 nL s-1 and were spaced 5 mm apart. The plates were washed in methanol and activated for 5 minutes at 110°C prior chromatography. With a slit dimension of 5 mm 0.45 mm and a data resolution of 100 m step-1, the scanning speed was 20 mm s-1. Each track was scanned three times with baseline correction, with the monochromatic band width set at 258 nm.[14]

The mobile phase was toluene: methanol: ammonia (30 percent) (7.5: 2: 0.5 v/v/v), with 10 mL utilised per chromatographic run. In a saturated mobile phase 20 cm x 10 cm twin trough glass chamber, linear ascending development was carried out (Camag , Muttenz , Switzerland). The optimal chamber saturation time for the mobile phase was 20 minutes at room temperature (25°C 2) and 60% relative humidity. Each chromatogram was created over an 80-mm distance. The TLC plates were dried in a wooden chamber with adequate ventilation in a stream of air using a hair drier upon development. Sitometric examination was performed at 258 nm. The radiation source was a deuterium lamp with a continuous UV spectrum between 200 and 400 nm. The analysis was done by linear regression of UV absorption peak areas as a function of sample analysis.

1.3.1 The samples were spotted in the form of 3 mm wide bands on precoated silica gel aluminium plate 60 F254 using a Camag Linomat IV and a Camag microlitre syringe (E. Merck, Germany).

(Switzerland). The mobile phase of toluene, ethyl acetate, methanol, and triethylamine in the ratio of 7:2:1:0.1 v/v/v/v. The plates were prewashed in methanol and activated at 60°C for 5 minutes prior to chromatography. Samples were laid out and in 3 mm long strips at 5 mm intervals under a spray of nitrogen. Slit dimensions was 3 0.1 mm. After the mobile phase had been saturated for 30 minutes, linear ascending chromatogram development to a distance of 8 cm was completed in a twin trough TLC developing chamber (Camag) at room temperature. After just being produced, TLC plates were dried in a stream of air using an air dryer. Densitometric scanning was performed using a Camag TLC scanner III in absorbance mode at 210 nm. The source of radiation was a deuterium lamp.

II. Stationary Phase:

HPTLC is a type of recent TLC that is the most advanced. HPTLC plates with microscopic particles with a small particle size are used, resulting in homogeneous layers with a smooth surface. In HPTLC, smaller plates (10×10 or 10×20 cm) are used. In industrial pharmaceutical densitometric quantitative analysis, HPTLC plates are used because they provide better resolution, detection sensitivity, and in situ quantification. In more than 90% of reported pharmaceutical and drug evaluation, normal phase adsorption TLC on silica gel with a less polar mobile phase, such as chloroform–methanol is used. [15]

1. 1. HPTLC methods for simultaneous estimation of two anti-inflammatory drugs were established that are simple and precise (curcumin and galangin). The approach was developed with the intent of examining both medications in their commercial dose form (capsules) with limited impact from components. On precoated TLC plates, chromatographic separation was performed using n-hexane, ethyl acetate, acetic acid, and methanol as the mobile phase (60 F254, 20 cm x 10 cm, 250 m thickness, Merck, Darmstadt, Germany). At 404 nm, spectrodensitometric analysis was used to detect and quantify the presence of the substance. [16]
2. The TLC densitometric method was established and validated for quantification of stigmasterol from petroleum ether extract of Bryophyllumpinnatum leaves and stems. TLC aluminium plates precoated with silica gel 60 F254 were used to separated the samples. As in mobile phase, Chloroform: Ethanol (9.8:0.2 v/v) provided good separation. To determine and quantify, densitometric scanning in the reflection/absorbance mode at 490 nm was used. [17]
3. It explains how to estimate it using a simple, precise, and accurate HPTLC approach in bulk and tablet dose form. Using a mobile phase of methanol and toluene (4:3 percent v/v) and spot densitometry at 235nm, chromatographic separation was performed on precoated silica gel 60 F254 aluminium plates. [18]

Lipophilic C-18, C-8, and C-2 silica gel phases, as well as phenyl chemically modified silica gel phases and hydrocarbon-impregnated silica gel plates made with a more polar aqueous mobile phase, such as methanol–water or dioxane–water, are for reversed-phase TLC. Lipophilic C-18, C-8, and C-2 silica gel phases, as well as phenyl chemically modified silica gel phases and hydrocarbon-impregnated silica gel plates made with a more polar aqueous mobile phase, such as methanol–water or dioxane–water, are used for reversed-phase TLC.

1. Minocycline in human plasma can now be determined using a new high-performance thin-layer chromatographic (HPTLC) method. Chromatograph with a mobile phase of 5:4:0.5:0.5 (v/v) methanol, acetonitrile, isopropanol, and water on aluminium plates coated with silica gel 60F254. Densitometric analysis was performed at 345 nm. [19]
2. A simple, precise, accurate, and high-performance thin layer chromatographic approach has been devised and validated for the simultaneous detection of Olmesartanmedoxomil and hydrochlorothiazide combined dose forms. As

the stationary phase, precoated silica gel 60F254 had been used. The mobile phase was made up of acetonitrile, chloroform, and glacial acetic acid (7:2:0.5, v/v/v). At a wavelength of 254nm, the spots were detected. [20]

3. For the measurement of tenoxicam in the Sonia K et al /J. Pharm. Sci. & Res. Vol. 9(5), 2017,652-657 micro emulsion gels, a simple, precise, accurate, and rapid high performance thin layer chromatographic approach has been devised and validated. As a stationary phase, tenoxicam was chromatographed on a silica gel 60 F254 TLC plate. Toluene: ethyl acetate: formic acid (6:4:0.3 v/v/v) was utilised as the mobile phase. [21]
4. A simple, precise, specific, and accurate high performance thin layer chromatographic approach has been devised for the simultaneous detection of Cinitapride and Omeprazole in medicinal dose form. On Merck HPTLC aluminium plates of silica gel G60 F254, (20 x 10 cm) with 250m thickness, the separation was done using chloroform: ethyl acetate: methanol (7.3: 2: 0.7, v/v/v) as the mobile phase. HPTLC separation of the two medicines was conducted in the absorbance mode at 277 nm, followed by densitometric measurements. [22]
5. It discusses a tested thin-layer liquid chromatography (TLC) method for estimating telmisartan and ramipril in a combination dose form. This procedure does not require the separation of components from the sample. In order to determine telmisartan and ramipril in tablet dose form, high performance thin layer chromatography (HPTLC) was used. A TLC-precoated silica gel on an aluminium plate 60 F 254 (10 cm 10 cm), prewashed with methanol and activated at 60° C for 5 minutes before chromatography, was used in the procedure. The solvent system used was acetone: 5:3:2:0.03, (v/v/v/v) Ethyl acetate: Glacial acetic acid: Benzene [23]

Other pre-coated layers used aluminumoxide, magnesium silicate, magnesium oxide, polyamide, cellulose, kieselguhr, ion exchangers, and polar modified silica gel layers with linked amino, cyanodiol, and thiol groups. A chiral layer made of C-18 is being used to separate optical isomers.

III. Mobile Phase:

The stationary phase's adsorbent material, as well as the analyte's physical and chemical properties, are used to determine the mobile phase. Because of their diverse selectivity properties, diethyl ether, methylene chloride, and chloroform, individually or in combination with hexane as the strength adjusting solvent for normal-phase TLC, and methanol, acetonitrile, and tetrahydrofuran mixed with water as the strength adjusting solvent for reversed-phase TLC, are the mobile-phase systems used. A mobile phase containing 25 mM sodium pentane sulfonate in methanol–0.1 M acetate buffer is used to perform ion pairing separations on C-18 layers (pH 3.5). (15.5:4.5).

1. Simultaneous quantification of two anti-inflammatory medicines was accomplished using simple and precise HPTLC methods (curcumin andgalangin). The technique was created to evaluate both drugs in their commercial dosage form (capsules), with no other substances interfering. Chromatographic separation was performed on precoated TLC plates (60 F254, 20 cm 10 cm, 250 mm thickness) using n-hexane, ethyl acetate, and a linear ascending approach (Merck, Darmstadt, Germany). Acetate, acetic acid, and methanol make up the mobile phase. [16]
2. For simultaneous quantification of Lamivudine and Zidovudine in tablets, an HPTLC method was developed and validated. The chromatograms were produced on a pre-coated plate of silica gel GF aluminium TLC plate with a mobile phase of toluene: ethyl acetate: methanol (4:4:2, v/v/v) and quantified using densitometric absorbance mode at 276 nm. [24]
3. Minocycline in human plasma can be detected to use a new high-performance thin-layer chromatographic (HPTLC) method. The mobile phase was methanol: acetonitrile: isopropanol: water 5:4:0.5:0.5 (v/v) and the chromatography was done on aluminium plates coated with silica gel 60F254. [19]
4. The quantitative quantification of Eugenol in muscle and joint pain relaxant herbal oil was devised and confirmed using a new and simple HPTLC approach. The plates were TLC aluminium plates that were precoated with 60F-254 silica gel (0.2 mm thickness). A linear ascending development was carried out in a twin trough glass chamber saturated with mobile phase. The Toluene: Ethyl acetate (9.3:0.7) ratio is found using a TLC scanner (CAMAG) in reflectance/absorbance mode at 560 nm, followed by densitometric measurement. [25]
5. A sensitive, quick, and repeatable high performance thin layer chromatographic approach for simultaneous detection of diosgenin and quercetin from fenugreek seeds has been devised using TLC aluminium plates precoated with silica gel G60F254.The best separation was achieved using a mobile phase combination of Toluene-ethyl acetate-formic acid (5:4:1, v/v/v). Densitometric scanning of the plates at 275nm was used to determine quercetin levels. [26]
6. A new, simple, and rapid thin-layer chromatographic approach was developed and validated for the quantitative detection of carbamazepine. Carbamazepine was chromatographed on a silica gel 60 F254 TLC plate with a mobile phase of ethyl acetate: toluene: methanol (5.0: 4.0: 1.0 v/v/v). Carbamazepine was measured by densitometric analysis at 285 nm. [27]
7. A new easy, precise, accurate, specific, and selective high performance thin layer chromatographic (HPTLC) technique was devised for the simultaneous measurement of Terbinafine hydrochloride (TH) and Mometasonefuroate (MF) in cream dosage form. On Merck precoated silica gel aluminium plate 60 F254, chromatographic separation was achieved using Toluene: Ethyl acetate: Glacial acetic acid (8: 4: 0.1 v/v) as the mobile phase. [28]
8. . For the qualitative and quantitative examination of diclofenac sodium tablets, a Thin Layer Chromatography (TLC) method was designed and validated in line with ICH and USP criteria. The method was developed on pre-coated TLC silica gel 60 F254 glass plates in the reflectance absorbance mode with a saturation time of 25 mins and a

densitometer detection wavelength of 284 nm, using a mobile phase prepared with environmentally friendly solvents toluene, acetone, and glacial acetic acid (10:15:0.2 v/v/v). [29]

9. An accurate, sensitive, precise, dependable, and rapid high-performance thin layer chromatography method for determining cholesterol content is created. J. Pharm. Sci. & Res., 9(5), 652-657, 2017. Sonia K & her colleagues The samples were sprayed with the CAMAG sample applicator Linomat 5 during the stationary phase of this method, as used aluminium-backed pre-coated silica gel 60 F254 plates. A mobile phase of chloroform/methanol (9.5:0.5, v/v) was used to create the chromatogram. [30]

10. Mycophenolate mofetil in bulk and tablet dose forms can be evaluated using a new, simple, high-performance thin-layer chromatographic method. On aluminium plates precoated with silica gel 60 F254, toluene, acetone, and methanol in the ratio of 6:2:2 (v/v/v) were used as the mobile phase. Quantitative analyses were performed using densitometric scanning at 254 nm. [31]

11. A HPTLC method for estimating duloxetine hydrochloride in bulk and tablet dose form that is simple, precise, and accurate. The chromatographic separation was performed out on precoated silica gel 60F254 aluminium plates with a mobile phase of chloroform: methanol (8:1 v/v) and spot densitometry at 235nm. [32]

3.2. Optimization of the mobile phase:

Various solvent systems, such as a mixture of (a) toluene: methanol (7:3 v/v): (b) triethylamine : methanol (6: 3 v/v) (c) To separate and resolve the spot of Lacosamide from its impurities and other formulation excipients, toluene: chloroform: methanol (1: 5: 3 v/v/v) and (d) toluene: ethyl acetate: methanol (0.5: 4: 2 v/v/v) were used**.** Lacosamide was resolved in a 7.5: 2 v/v/v combination of toluene: methanol, however there was tailing in the peaks. 30 percent ammonia was added to increase peak symmetry. Finally, a 30 percent mixture of toluene: methanol: ammonia (7.5: 2: 0.5 v/v/v) produced a well-resolved peak with improved peak shape. (Rf = 0.240.02) was used to dissolve the medication. The TLC chamber was pre-saturated with mobile phase for 20 minutes, which ensured greater repeatability and resolution in Lacosamide migration.

3.2 Prewashing:

Plates are handled at the upper edge to avoid contamination. Plates are used without preparation unless chromatography creates impurity fronts due to plate contamination. For repeatability and quantitative analysis, layers are frequently prewashed with 20 mL methanol. Methanol, a mixture of methanol and ethyl acetate, or even mobile phase are used as prewashing solvents per trough in a 20 x 10 cm twin trough chamber (TTC). The TTC's trough can create two 20 10 cm plates or four 10 10 cm plates back-to-back. Remove the plate and dry it in a clean drying oven for 20 minutes at 120°C. In a suitable container with dust and fume protection, acclimate the plate to the laboratory atmosphere (temperature, relative humidity).

3.3. Preparation of plate:

With the correct equipment, TLC plates can be made. These layers don't adhere well to the glass. Most of the restrictions of a produced layer are overcome by using small amounts of very high molecular weight polymer as a binder in precoated plates. Abrasion-resistant precoated layers have a constant layer thickness, are reproducible, and are preactivated and ready to use. Glass, metal, or polyester can be used to support them. Purchased aluminium foil plates are less expensive, may be sliced, and are thus easier to handle, transport, or mail. For the greatest results, glass plates are the best option. Layers containing the fluorescent indication F 254 are most commonly utilised. This makes it possible to visualise samples in a UV cabinet quickly, easily, and without causing damage. In TLC, the most common plate size is 2020 cm, while in HPTLC, the most common plate size is 20 ×10 cm or 10 × 10 cm.

IV. HPTLC procedure's steps are as follows:

4.1 Sample Application

4.2 Chromatogram Development

4.3 Derivatization

4.4 Evaluation Detection

4.5 Evaluation Documentation

4.1 Sample Application:

The application of samples is critical, and spot application and spraying on samples are two ways used. .In chromatography, the application of the sample is the first step, and it has an impact on the end product's quality. The method of application and equipment to employ are defined by the requirements. The simplest way is to use a set volume capillary to apply a sample spot by spot. Samples quantities of 0.5 to 5 L can be placed as spots on standard layers without drying; sample volumes of up to 1 L per spot are allowed on HPTLC layers. The best resolution may be achieved

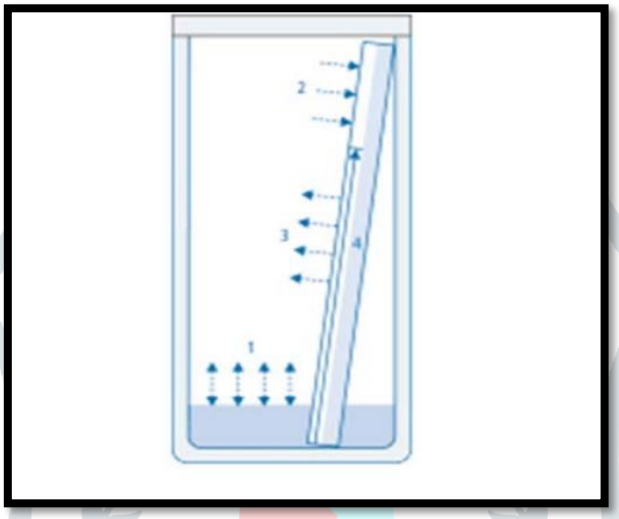
using the chromatographic approach by spraying small bands of larger quantities on samples. Big sample quantities or materials contains lots of matrices can be sprayed on and targeted into narrow bands in the size of rectangles.

4.2 Developing Chromatograms:

In add to the static and mobile phases, this technique includes a gas phase. This gas phase has a significant influence on the separating outcome.

Processes:

The lower level of the plate should be immersed, and the developing solvent should flow up the layer by capillary effect until the necessary range is reached and the chromatography is halted. The following points mostly involve silica gel as a stationary phase and advancements known as adsorption chromatography.



There are four different types of processes that occur.

1. When the stationary phase is dry, it adsorbs molecules from the gas phase. Adsorptive saturation is a balancing act in which polar components from the gas phase are withdrawn and loaded onto the surface of the stationary phase.
2. The gas phase reacts with the mobile phase-wetted region of the layer. As a result, the liquid's less polar components are released into the gas phase. J. Pharm. Sci. & Res., Vol. 9(5), 2017,652-657, Sonia K et al

4.3 Choosing a developing chamber:

During technique development, the chamber is chosen based on factors such as which chamber is available and which must be employed. Economic considerations such as time and solvent consumption should also be considered. For development, a typical 20 x 10 cm Twin Trough Chamber is used. The chamber geometry and chromatographic parameters of existing analytical methods can be kept, while environmental and operational implications can be harmonized.

V. Result and Discussion:

5.1. Evaluation: Documentation, TLC-MS,

Bioluminescence:

Capturing and archiving electronic pictures for documentation is simple. They can be compared to current photos by being reproduced on screen without changing over time. TLC-MS and bioluminescence are two technologies that enhance TLC's capabilities.

5.2. Application:

5.2.1. Application of Pharmaceuticals:

Quality control

Content Uniformity Test (CUT)

Identity- and purity checks

Stability tests, etc.

5.2.2. Application in the clinic:

Lipids

Metabolism studies

Drug screening

Doping control etc.

5.2.3. Application of cosmetics:

Identity of raw material

Preservatives,

Coloring materials, etc.

Screening for illegal substances, etc.

5.2.4. Botanical dietary supplements and herbal medications:

Classify

Adulteration detection

Checks for stability tests, etc.

Detection of marker chemicals/compounds

5.2.5. Food and feed supplies

Controlling the quality

Additives (e.g. Vitamins)

Checks for stability tests (expiration), etc.

5.2.6. Applications in industry:

Developing and improving processes

Monitoring of the process

Validation of cleanliness, etc

5.2.7. Forensics:

Document forgery detection

The poisoning investigation

Analyses of Dyestuff, etc

5.3. Evaluation for high precision requirements

1.HPTLC plates were used in this evaluation. A thin layer, a narrow particle variation in size, and homogenous layer of HPTLC packing are used to achieve less fraction broadening and minimal background noise.

2.An automated spray-on sample application approach. The size of the beginning zone remains independent of the application volume and the sample is spread uniformly over the application position only when spraying. Data can be collected at higher drug levels.

3.The use of a chamber with high repeatability of chamber conditions is recommended.

4.Selecting a calibrated operating range based on the compounds' absorption and fluorescence characteristics. Calibrating functions are available in the evaluation process.

5.Slit dimensions, measuring wavelength, and scanning speed are all factors to consider when optimizing light and measurement settings for the compounds to be examined.

6. Appropriate baseline correction to improve signal-to-noise ratio.

7. Derivatization can add to the overall inaccuracy of the judgment. The lesser the mistake, the more homogeneous the reagent is applied.

Conclusion:

A simple, selective, precise, and reproducible high-performance thin-layer chromatographic (HPTLC) method for the analysis of Lacosamide in bulk drug and tablet dosage form was developed. Statistical analysis of the obtained data showed the selectivity of the proposed method for Lacosamide estimation as a bulk drug and tablet dosage form. The present investigation demonstrated that the developed methods were successfully applied for pre- clinical and clinical studies.

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Fig.No.1)

SELECTION OF DETECTION WAVELENGTH BY HPTLC

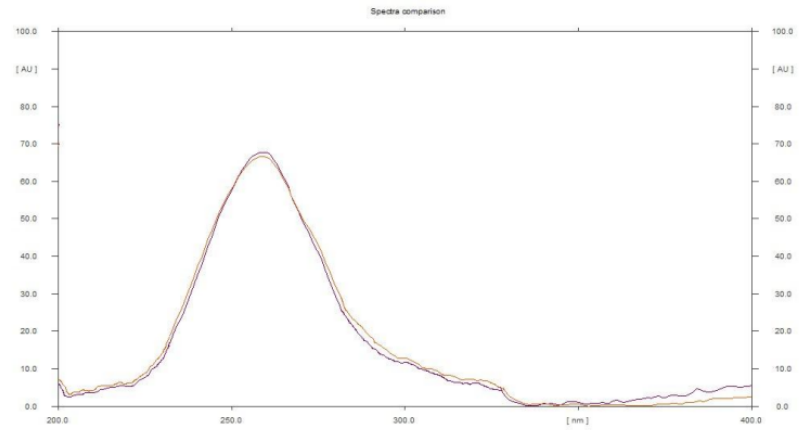

Fig.No.2)

LINEARITY CHROMATOGRAM OF LACOSAMIDE BY HPTLC (1 µg/ mL)

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.64	0.1	0.69	33.6	46.85	0.72	5.3	1059.1	36.33	Lac

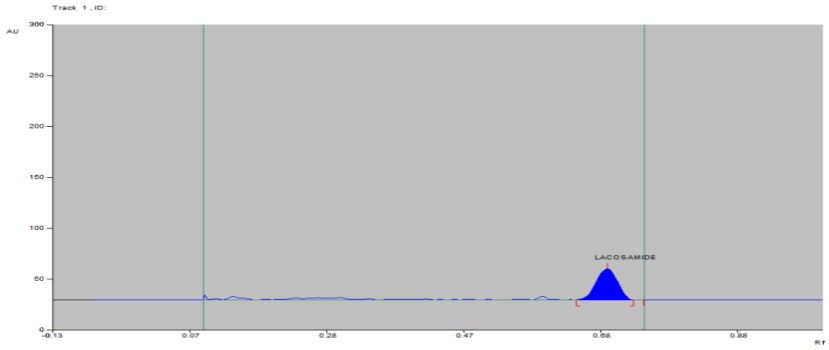

Fig No.3)

LINEARITY CHROMATOGRAM OF LACOSAMIDE

BY HPTLC (2 µg/ mL)

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.61	0.0	0.66	75.4	100.0	0.75	0.69	2146.3	100.00	Lac

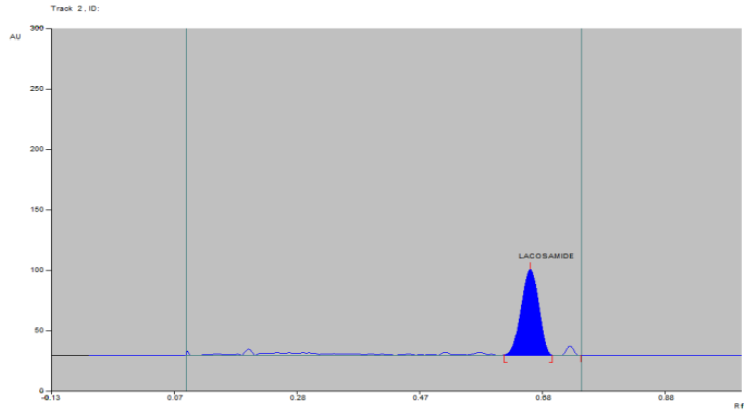

Fig No. 4)

LINEARITY CHROMATOGRAM OF LACOSAMIDE

BY HPTLC (3 µg/ mL)

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.60	0.6	0.66	102.1	100.00	0.70	7.6	2970.3	100.00	Lac

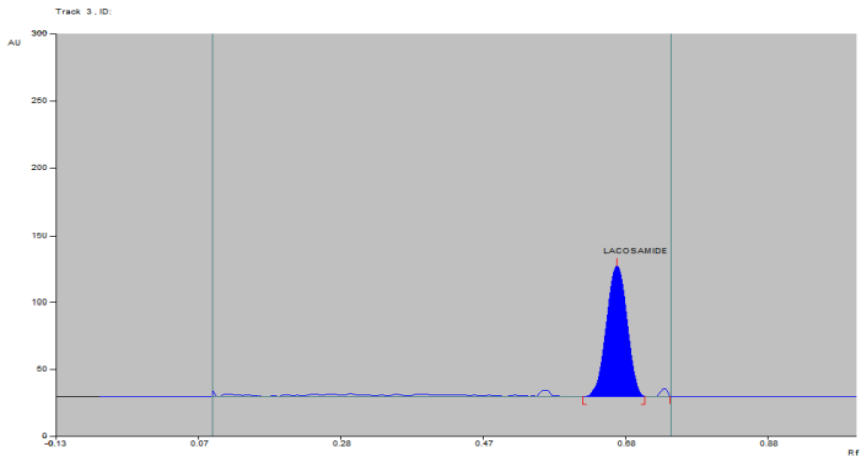

Fig.No. 5)

LINEARITY CHROMATOGRAM OF LACOSAMIDE

BY HPTLC (4 µg/ mL)

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.62	2.5	0.67	128.9	100.00	0.71	10.8	3864.9	100.00	Lac

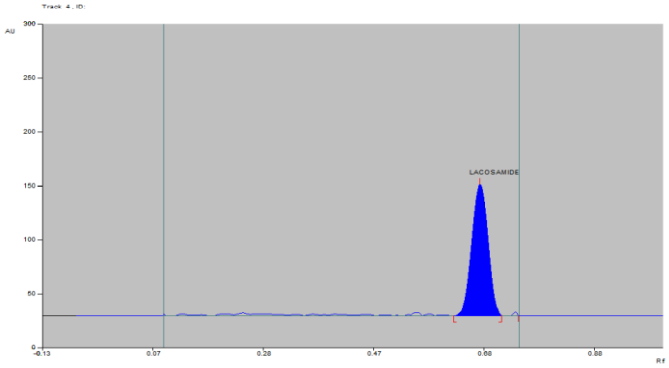

Fig No. 6)

LINEARITY CHROMATOGRAM OF LACOSAMIDE

BY HPTLC (5 µg/ mL)

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.61	3.6	0.67	128.9	100.00	0.71	10.8	3864.9	100.00	Lac

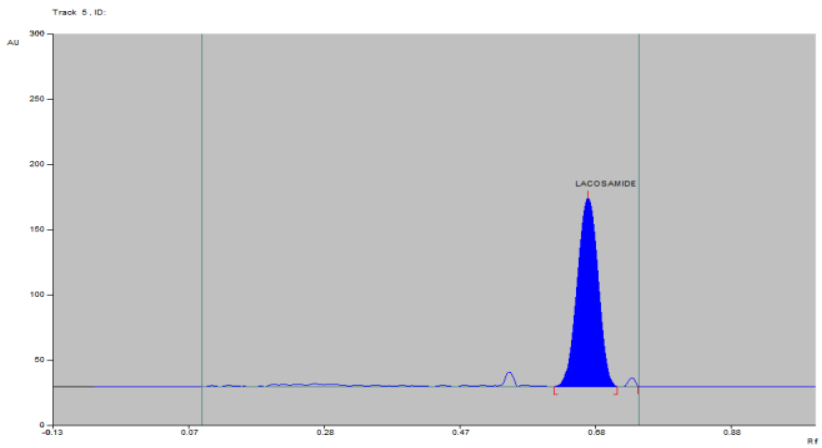

Fig no. 7)

LINEARITY CHROMATOGRAM OF LACOSAMIDE

BY HPTLC (6 µg/ mL)

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.60	3.3	0.66	180.6	100.00	0.70	16.5	5669.0	100.00	Lac

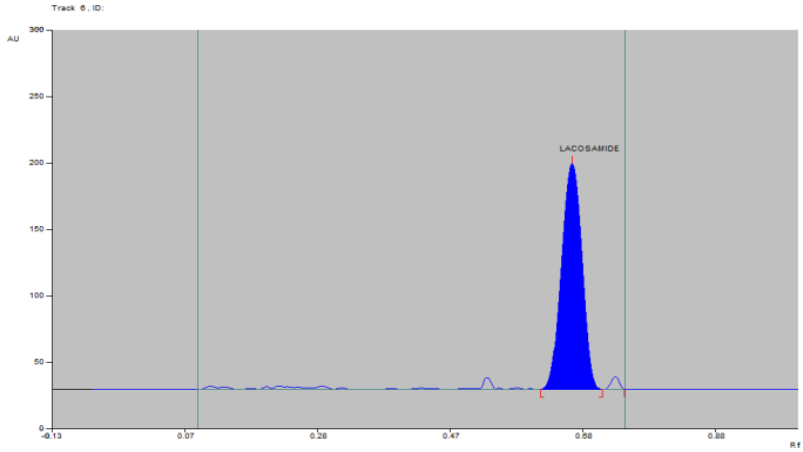

Fig No. 8)

CALIBRATION CURVE OF LACOSAMIDE

BY HPTLC

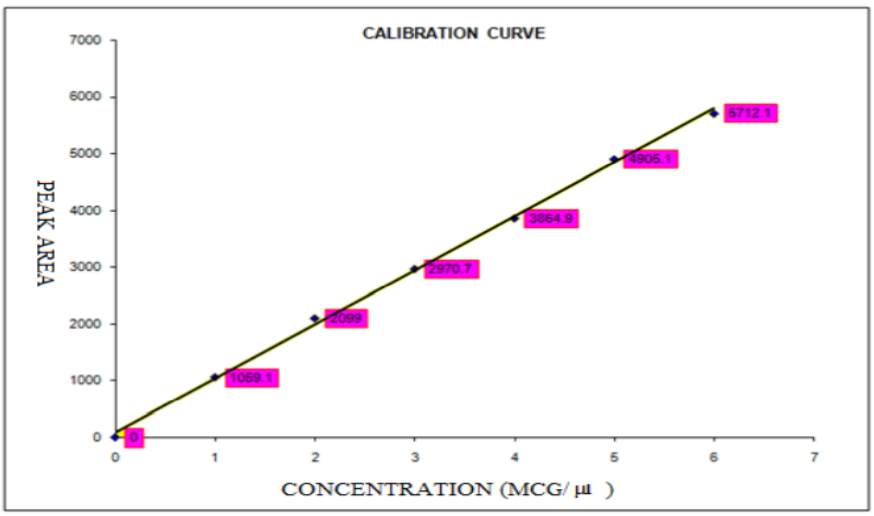

Fig No. 9)

CHROMATOGRAM FOR THE ANALYSIS OF LACOSAMIDE FORMULATION (LACOSAM) REPEATABILITY-1

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.63	0.2	0.67	128.9	100.0	0.70	1.0	2821	100.00	Lac

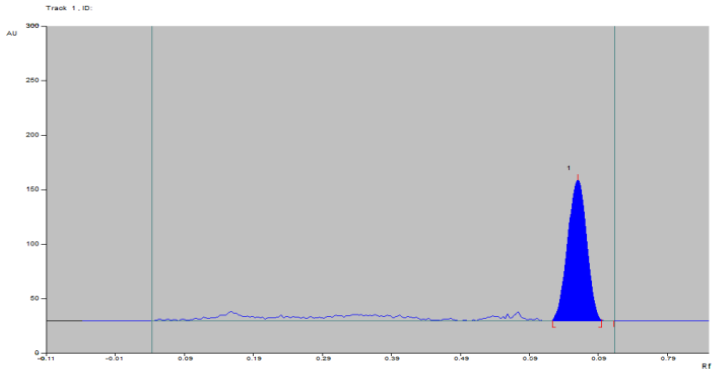

Fig No. 10)

CHROMATOGRAM ANALYSIS FOR THE OF LACOSAMIDE FORMULATION (LACOSAM) REPEATABILITY-2

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.61	0.0	0.66	75.4	100.0	0.75	0.69	2970	100.00	Lac

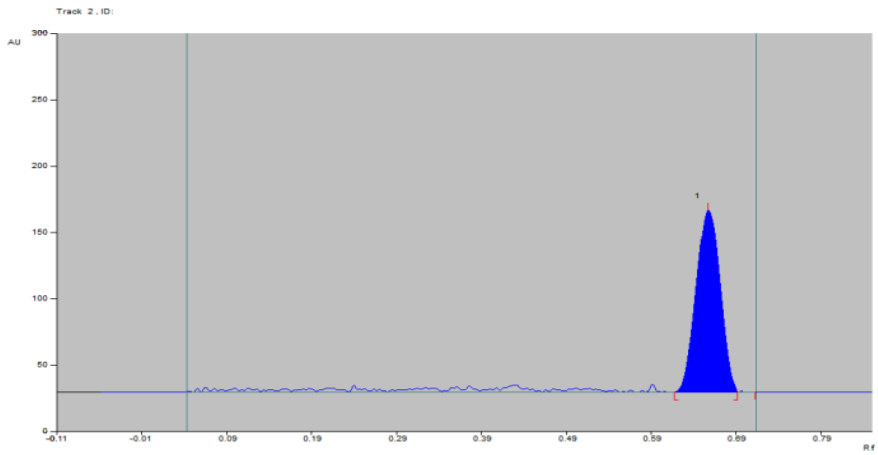

Fig No. 11)

CHROMATOGRAM ANALYSIS FOR THE OF LACOSAMIDE FORMULATION (LACOSAM) REPEATABILITY-3

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.62	0.0	0.66	137.1	100.0	0.70	0.6	2906.9	100.00	Lac

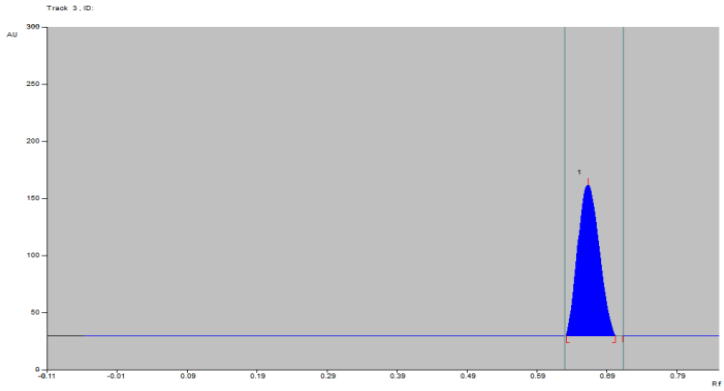

Fig No. 12)

CHROMATOGRAM ANALYSIS FOR THE OF LACOSAMIDE FORMULATION (LACOSAM) REPEATABILITY-4

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
30.67	5.9	0.67	132.3	78.86	0.71	0.4	2959.6	100.00	Lac

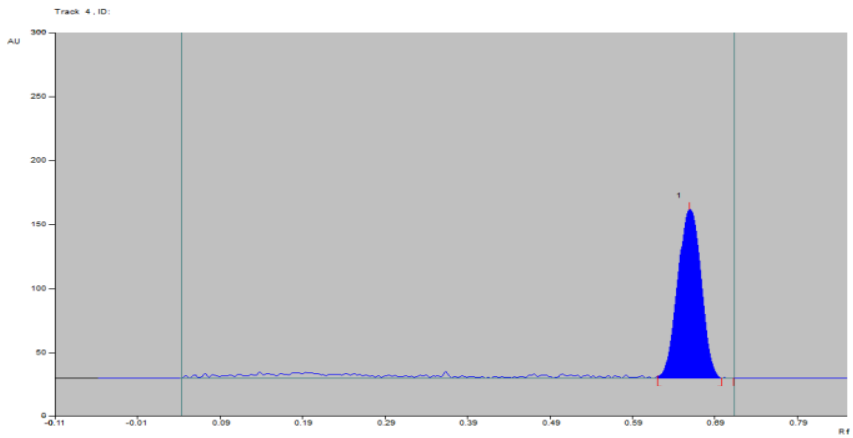

Fig No. 13)

CHROMATOGRAM ANALYSIS FOR THE OF LACOSAMIDE FORMULATION (LACOSAM) REPEATABILITY-5

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.63	1.5	0.66	132.7	100.0	0.70	0.0	2945	100.00	Lac

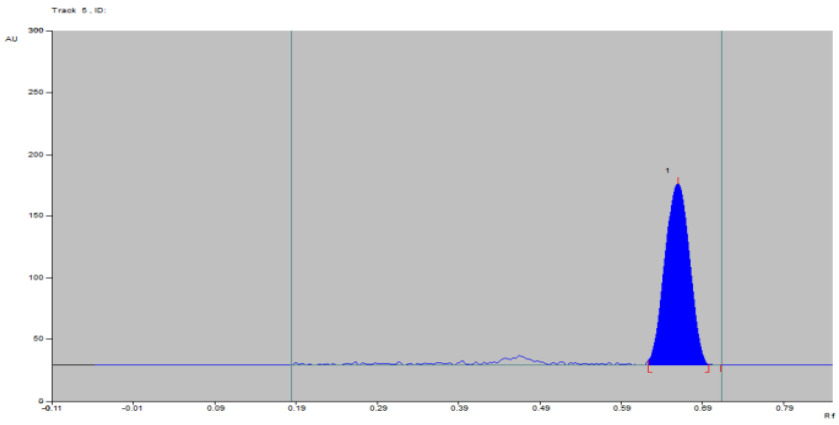

Fig No. 14)

CHROMATOGRAM ANALYSIS FOR THE OF LACOSAMIDE FORMULATION (LACOSAM) REPEATABILITY-5

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.63	1.5	0.66	132.7	100.0	0.70	0.0	2945	100.00	Lac

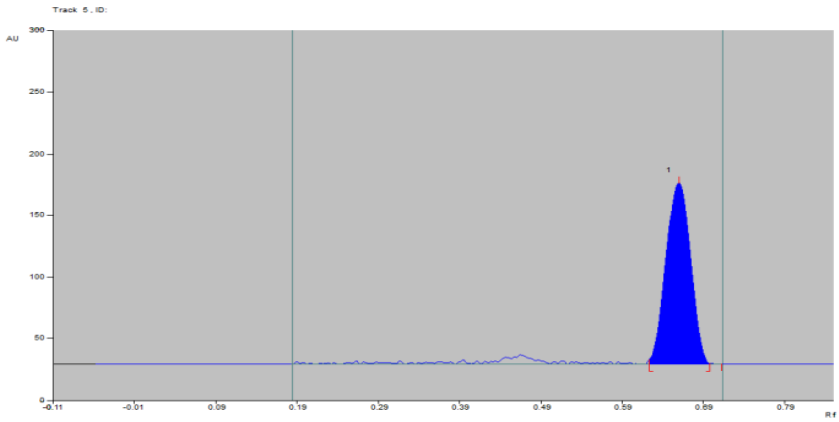

Fig No. 15)

CHROMATOGRAM FOR FIRST RECOVERY ANALYSIS OF FORMULATION (LACOSAM) BY HPTLC

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.61	0.0	0.66	75.4	100.0	0.75	0.69	3267	100.00	Lac

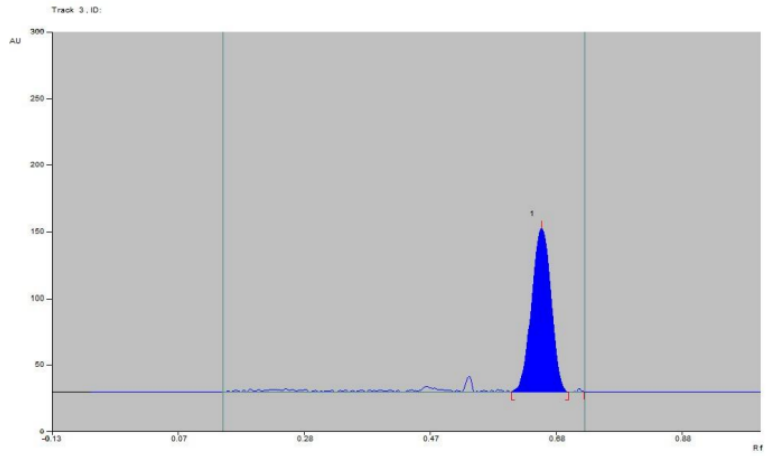

Fig No.16)

CHROMATOGRAM FOR SECOND RECOVERY ANALYSIS OF FORMULATION (LACOSAM) BY HPTLC

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.61	0.0	0.66	75.4	100.0	0.75	0.69	3564	100.00	Lac

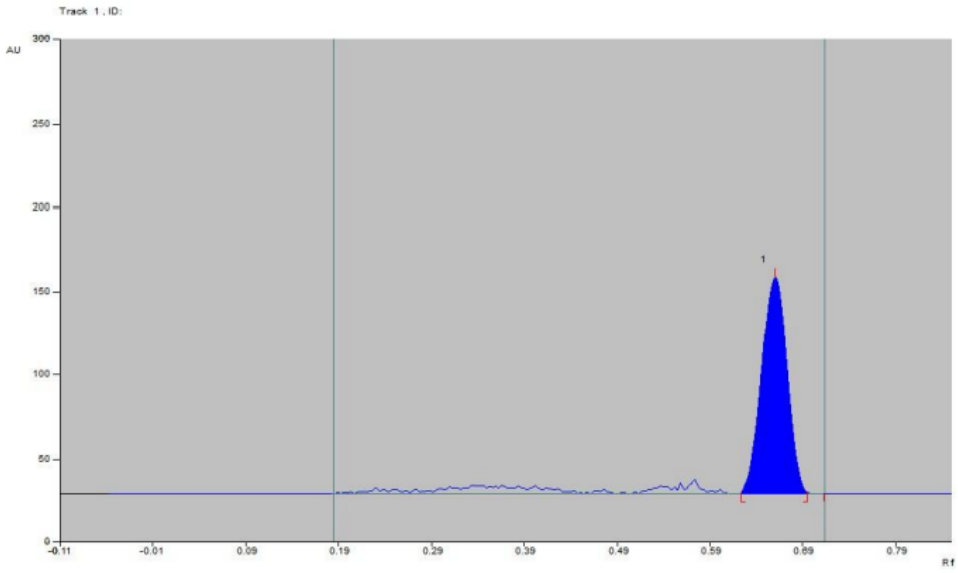

Fig No.17)

CHROMATOGRAM FOR THIRD RECOVERY ANALYSIS OF FORMULATION (LACOSAM) BY HPTLC

Peak	Start Rf	Start Height	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
10.61	0.0	0.66	75.4	100.0	0.75	0.69	3861	100.00	Lac

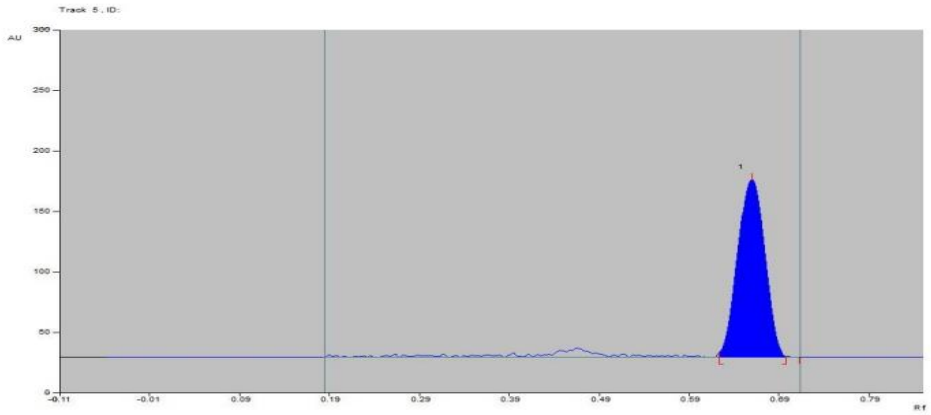

TABLE: 1)

OPTICAL CHARACTERISTIC OF LACOSAMIDE BY HPTLC METHOD

S.NO	PARAMETERS	VALUES
1	λmax (nm)	257 nm
2	Beers law limit (ng/mL)	1000-6000
3	Correlation coefficient (r)	0.9992
4	Regression equation (y=mx+c)	Y=954.79X+97.63
5	Slope (m)	954.79
6	Intercept (c)	97.63
7	LOD (µg/mL)	0.0540
8	LOD (µg/mL)	0.1638
9	Sandell s sensitivity (µg/cm^2 0.001A.U)	1.047
10	Standard error of mean	2.3559

TABLE: 2)

QUANTIFICATION OF FORMULATION (LACOSAM) BY HPTLC METHOD

S.No	Labeled Amount	Amount Found	Percentage Obtained	Avg %	S.D	% R.S.D	S.E
1	50 mg	49.56 mg	99.13	99.13	1.1737	1.1840	0.03260
2	50 mg	49.23 mg	99.47				
3	50 mg	50.19 mg	100.38				
4	50mg	50.13 mg	100.26				
5	50mg	48.70 mg	97.40				
6	50mg	49.08 mg	98.16				

★mean of six observation

TABLE: 3)

INTRADAY PRECISION ANALYSIS OF FORMULATION (LACOSAM) BY HPTLC METHOD

Drug	S.No	Labeled amount (mg/tab)	Amount found (mg/tab)	Percentage obtained	Average percentage	S.D	% R.S.D	S.E
Lac	1	50	49.08	98.16	98.30	0.3442	0.3502	0.0382
	2	50	49.03	98.06				
	3	50	49.39	98.70				

★mean of three observation

Table: 4)

INTERDAY PRECISION ANALYSIS OF FORMULATION

(LACOSAM) BY HPTLC METHOD

Drug	S.No	Labeled amount (mg/tab)	Amount found (mg/tab)	Percentage obtained	Average percentage	S.D	% R.S.D	S.E
Lacosamide	1	50	49.39	98.78	99.54	1.4153	1.4218	0.1572
	2	50	50.59	101.18				
	3	50	49.34	98.68				

★mean of three observation

Table: 5)

RECOVERY STUDIES FORMULATION (LACOSAM 50 MG) BY HPTLC METHOD

Drug	%	Amount present (µg/mL)	Amount added (µg/mL)	Amt found	Amt recovery	% recovery	Avg % recovery	S.D	% R.S.D	S.E
Lacosamide	80	3.015	2.4	5.3932	2.3782	99.09				
	100	3.015	3.0	6.0161	3.001	100.00	100.36	1.687	1.679	0.187
	120	3.015	3.6	6.700	3.685	102.36				

★mean of three observation