Preformulation studies of Lamotrigine

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Abstract

The purpose of this study is to screen various preformulation studies in early stages of dosage form development, this study was to produce Nanoliposomes formulations containing lamotrigine (LAM) as interactive physical mixtures and nanosized LAM-based formulations. After risk assessment of the

Various preformulation studies were carried out including Preformulation Studies:- Evaluation Of Organoleptic Properties, Physical Characterization:- Melting Point, Differential Scanning Calorimetry (Dsc), X-Ray Diffraction (Xrd), U.V Spectral Analysis:- Determination Of Λ_{max} Of Lamotrigine, Preparation Of Calibration Curve Of Lamotrigine In Nasal Saline Buffer (Nsb; P^h 6.5):- A) Preparation Of Nasal Saline Buffer P^h 6.5. B) Preparation Of Calibration Curve Of Lamotrigine, Preparation Of Calibration Curve Of Lamotrigine In Methanol, Solubility Studies, Ltg Was Good Solubility In Methanol, determination Of Partition Coefficient Of Ltg, Fourier Transform Infrared (Ftir) Spectroscopy, Quantitative Analysis Of Lamotrigine From Hplc (High Performance Liquid Chromatography) Method: Instrumentation, Preparation Of Standard Stock Solution, Linearity (Calibration Curve), Mobile Phase , Preparation Of Stock Solution, Preparation Of Sub Stock Solution, Preparation Of Different Solution.

The nasal delivery of drugs offers a great alternative route to avoid adverse events and to increase patient compliance due to its advantageous properties. Besides nasal application, topical, systemic and central effects are also available.

Keywords: preformulation, brain targeting, lamotrigine, nasal drug delivery.
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Introduction

Preformulation involves application of biopharmaceutical principles to physicochemical parameters of drug substance with the goal of designing optimum drug delivery system in support of promising experimental formulations [1]. Its core objective lies in development of stable, effective and safe dosage form, since it generates useful information to the formulator to design an optimum drug delivery system [2]. Lamotrigine (LTG), is a well-tolerated anticonvulsant drug, approved for treatment of focal and generalized epilepsy in the elderly and in patients with epilepsies plus depressive disorders [3]. It undergoes extensive metabolism, and is mainly eliminated *via* the kidney [4]; where 94% of the orally administered dose is eliminated in urine and 2% in feces. Unchanged LTG accounts for 10% of the products detectable in urine [5]. Lamotrigine is a second-generation antiepileptic drug of the phenyltriazine class which is used widely in epilepsy, simple and complex seizures, generalized tonic-clonic seizures, and bipolar that shows a broad spectrum of actions against partial, generalized tonic-clonic seizures and Lennox-Gastaut syndrome either alone or as adjunctive

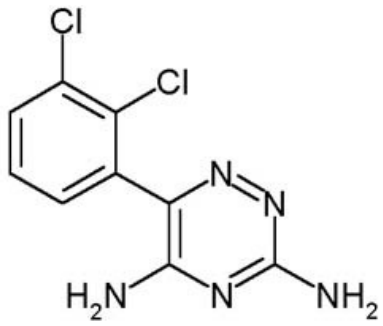
therapy [6, 7]. Comparatively, lamotrigine shows relatively few side effects and does not require blood monitoring in monotherapy [8, 9]. However, peak plasma concentration reaches after 1.4 h its oral administration. This delay in the onset of action in spite of good bioavailability is due to its low aqueous solubility (0.17 g/l) [2,8]. Hence it is required to improve the solubility and dissolution of lamotrigine.

Lamotrigine

CAS NO: 84057-84-1

Chemical name: 6-(2,3-Dichlorophenyl)-1,2,4-triazine-3,5-diamine

Chemical structure



Synonyms : Lamictal, **Lamictal** CD, **Lamictal** Odt, **Lamictal** XR.

Molecular Formula : C9H7Cl2N5

Molecular Weight : 256.09 g/mol

FDA Drug class: Phenyltriazine class

Category : Standards; Pharmaceutical/API Drug, Anti-epileptic drug

Subcategories: Second generation antiepileptic drug

BCS Classification: Class 2: Low Solubility, High Permeability.

Physical Description : Solid

Appearance: White to pale cream-colored powder

Melting Point: 216-218 °C

Odor: Odorless

Boiling Point: 503.1±60.0

Solubility: Sparingly soluble in methanol, Soluble in ethanol, Less soluble in water

In water, 170 mg/L at 25 °C

In 0.1M Hydrochloric acid: 4.1 mg/mL at 25 °C

PKa: 8.53

Log P: 2.5

Bioavailability: 98%

Protein binding: 55%

Clearance: 0.18 to 1.21 mL/min/kg

Peak plasma concentration reaches at approx. 1-3 hr.

Dose: 25 mg/day (monotherapy)

Plasma half-life ($t_{1/2}$): 22.8 hr

Biological half-life: 24-34 hours

Mechanism of action: Various mechanisms of action exists for the drug lamotrigine for its diverse anti-seizure properties and psychotropic properties. Moreover Clinical profile study showed that

overlapping mode of action contribute to its pharmacological action. Clinical evidence of lamotrigine showed its potential effect against partial and secondarily generalize tonic-clonic seizures. Lamotrigine worked by blocking voltage-dependent membrane sodium channels by preventing excitatory neurotransmitter release. The drug also inhibits sodium dependent glutamate and aspartate release as well as γ-aminobutyric acid (GABA) release in cortical region. Lamotrigine binds to voltage- sensitive sodium channels and on serotonin 5- HT_3 receptors.

Distribution: Volume of distribution is 1.2 L/ Kg.

Metabolism: Lamotrigine is metabolized by the process of glucuronidation to form 2-N-glucuronide conjugate, which is pharmacologically inactive. Liver (UDP-glucuronosyltransferase 1–4, and UDP-glucuronosyltransferase 1–3 involving glucuronic acid conjugation)

Excretion: Excreted principally as a glucuronide conjugate from urine. Major amount of drug and its metabolites recovered in the urine and little recovered in the feces. Urine (65%), faeces (2%).

Therapeutic Uses: Anticonvulsants; Calcium Channel Blockers; Excitatory Amino Acid Antagonists; Voltage-Gated Sodium Channel Blockers

Pharmacokinetics : Lamotrigine follows first order kinetics. The absolute bioavailability of lamotrigine is 98%.

Adverse effects of lamotrigine: Skin rash, Insomnia , drowsiness, blurred vision, swelling of the face, anxiety, irregular breathing

Indications

Lamotrigine used as an supportive therapy for the seizures The drug can also be prescribed for those suffering from partial seizures belonging to minimum age of at least sixteen years

Drug Forms :

Form : Oral Tablet

Strength : 25mg.50mg.100mg.150mg and 200mg Form : Chewable Tablet

Strength: 2mg,5mg and 25mg Form : Orally Disintegrating Tablet

Strength : 25mg.50mg.100mg and 200mg Form :Extended Release Tablet

Strength : 25mg,50mg,100mg, 200mg,250mg and 300mg.

Available Brands:

Table 1::Lamotrigine is available in the market as tablet dosage form under the following Brand Names

Brand Name	Composition	Manufacturer
Lamepil Tab	Lamotrigine 25mg, 50mg	INNOVA(IPCA)
Lametec Tab	Lamotrigine 5mg, 25mg, 50mg, 100mg, 150mg, 200mg	CIPLA
Lamez OD tab	Lamotrigine 50 mg, 100mg, 150mg modified release.	INTAS
Lamidus -DT tab	Lamotrigine 50mg, 100mg	ZYDUS CADILA
Lemit Tab	Lamotrigine 50mg, 100mg	CONSERN PHARMA

Preformulation studies

Evaluation of organoleptic properties

The drug sample Lamotrigine (LTG) was examined for its nature, color, odor and taste.

Physical characterization

Melting point:

1. Powdering the compound and filling of capillary tube:

a) The thin walled capillary tube is sealed from one end by heating it on gas burner for a few seconds.

b) The given compound is crushed and it is pushed in the capillary tube in powdered form. The tube is repeatedly tapped and filled by the compound.

2. Fix the tube with thermometer:

a) The capillary tube is attached to the thermometer.

b) The whole apparatus is arranged.

c) The beaker in which the tube has to be placed is filled with paraffin or water.

3. Heating the liguid bath:

a) The liquid bath is heated gently with continuous stirring so that the constant temperature is maintained throughout the liquid using a glass loop stirrer moving up and down.

As the temperature reaches near the melting point of solid the flame is reduced and temperature is allowed to rise gradually.

4. Note the melting point:

a) Note the temperature when substance starts melting. The temperature is noted again when the solid melts completely.

The average or mean of two values gives correct melting point of the substance.

Differential Scanning Calorimetry (DSC):

Differential Scanning Calorimetry (DSC) of drug lamotrigine was recorded in differential scanning calorimetry (Perkin Elmer; Pyris 6 DSC, USA) to determine the transition temperature of drug.

X- Ray Diffraction (XRD):

XRD uses x-rays to investigate and quantify the crystalline nature of materials by measuring the diffraction of x-rays from the planes of atoms within the material. XRD spectrum was obtained and recorded using XRD Instruments.

U.V spectral analysis:

Determination of λ_{max} of Lamotrigine

The λ_{max} of Lamotrigine was determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer. Accurately weighed 10 mg of drug was dissolved in 10 ml of 6.5 pH phosphate buffer in 10 ml of volumetric flask.

Solubility studies:

Solubility study was performed in NSB, NSB with 10% methanol and neat methanol. Thesolubility of lamotrigine in various solvents was determined by adding an excess amount of drug in 2 ml of selected solvent in 5 ml capacity stoppered vials and mixed using a

vortex mixer. These vials were then kept at 25±1° C in an isothermal shaker for 72 hr to reach equilibrium. The equilibrated samples were removed from shaker and centrifugedat 3000 rpm for 15 min. The supernatant was filtered and diluted to required number of times with methanol if necessary and analyzed by UV spectrophotometer (13).

Determination of partition coefficient of LTG

The partition coefficient of the drug was determined by -Shaker Flask method. Excess of drug was added in equimolar mixture of 1-octanol and water inthe conical flask and kept in the mechanical shaker for 24 hr at 25ºC. The mixture was transferred in the separating fennel and allowed to stand for 24 hr to separate the water and octanol phase. After 24 hr the two phases were separated with the help of separating funnel. Both aqueous phase and octanol phase were filtered separately, diluted with methanol and absorbance was taken at its specific λmax of 306.5 nm by using Shimadzu UV-1601 spectrophotometer (14)**.** The partition coefficient of drug was determined by given formula:

Partition coefficient = Conc. $_{(Octanol)}$ / Conc. $_{(water)}$

Fourier Transform Infrared (FTIR) Spectroscopy:

FTIR spectroscopic analysis of LTG drug sample was carried out directly by putting in small hole of FTIR instruments (Bruker, Alpha T, Germany). The spectra were obtained between the UV range of 4000–400 cm^{-1}. The recorded spectra were compared with reference standard spectrum to analyze the characteristic peak of LTG from its molecular structure (15).

Quantitative Analysis Of Lamotrigine From HPLC (High Performance Liquid Chromatography) Method:

Mobile Phase

Acetonitrile: Methanol in the ratio of 50:50v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Preparation of Stock Solution

Accurately weighed 10 mg API of Lamotrigine was transferred into 10 ml volumetric flask and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

Preparation of Sub Stock Solution:

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (methanol) to give concentration of 100µg/ml of Lamotrigine respectively (Stock-B).

Preparation of Different Solution

0.1ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 10µg/ml for Lamotrigine.

Result and Discussion:

Table 2: Organoleptic properties of lamotrigine

S.No.	Parameters	Inferences
1.	Nature	Crystalline powder

2.	Color	White to pale cream-colored powder
3.	Odor	Odorless
4.	Taste	Bitter

Table 2: Determination of melting point

S.No.	Temperature when solid starts melting (t_1)	Temperature when solidification starts (t_2)	Average = $\frac{t_1+t_2}{2}$
1.	110^0c	106^0c	216^0c
2.	108^0c	110^0c	218^0c

The melting point of LTG was found at 216.80 ± 0.17º C by capillary method. Which was near to reported values (216-218 º C).

Differential Scanning Calorimeter (DSC):

Drug –Excipient compatibility study: Thermograms were recorded using a differential scanning calorimeter. Samples (5-10mg) were weighed and hermetically sealed in flat bottomed aluminium pans. These samples were heated over a temperature range of 50-400°C in an atmosphere of nitrogen (200ml/min) at a constant rate of 10^0C per minute, with alumina being the reference standard.

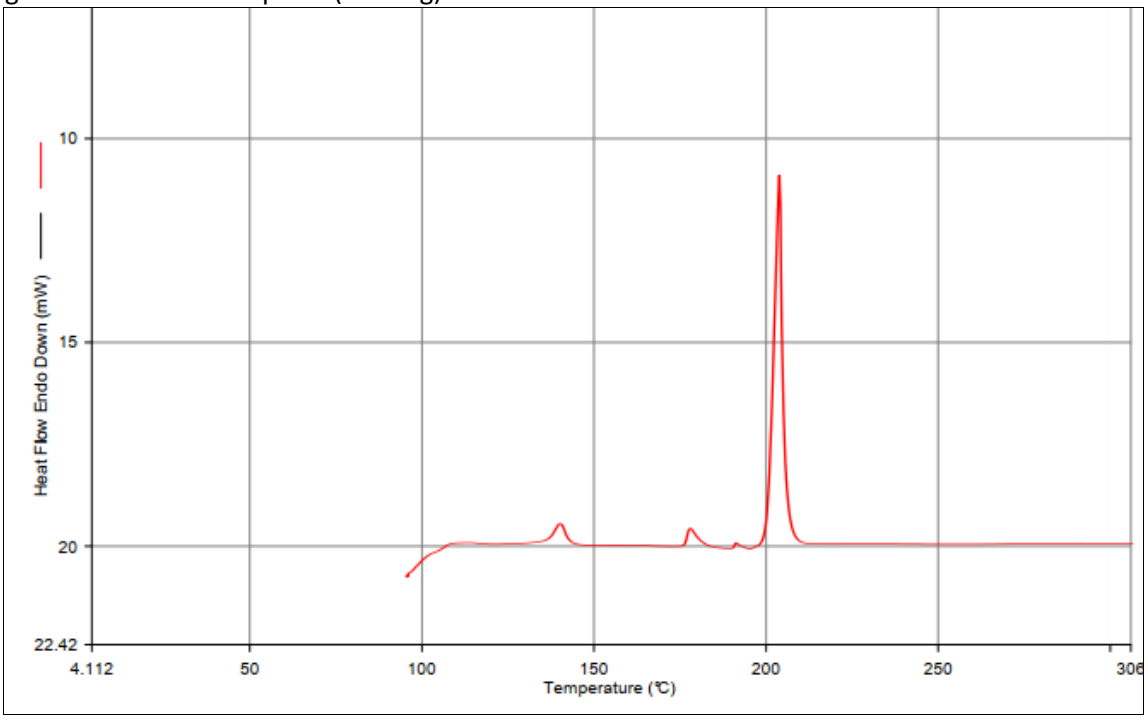


Figure 1: Thermogram of Lamotrigine

X- Ray Diffraction (XRD):

X-ray diffraction analysis of lamotrigine showed crystalline peak at different angles revealed the crystalline nature of the drug (Table 3).

Table 3: X-ray diffraction analysis of lamotrigine

Report	

Created on: Operator:	05-06-2022 14:22:03 SAILSOPS
File File name: File size (bytes):	 E:\XRD data\SIL-Lamotrigine.xrdml 84126
Time stamp Started: Finished: Total time (h:m:s):	 05-06-2022 17:34:50 05-06-2022 17:39:31 00:04:41
Comment	Pure Drug
Status	Completed
Measurement type	Single scan
Used wavelength Intended wavelength type: Kα1 (Å): Kα2 (Å): Kα2/Kα1 intensity ratio: Kα (Å): Kβ (Å):	 Kα1 1.540598 1.544426 0.50 1.541874 1.392250
Incident beam path **Radius (mm):** **X-ray tube Name:** Anode material: Voltage (kV): Current (mA): Focus Focus type: Length (mm): width (mm): Take-off angle (°):	 145.0 Empyrean Cu LFF HR (9430 0337310x) DK426454 Cu 40.0 7.5 Line 12.0 0.4 6.0
Soller slit Name: Opening (rad.):	Soller slits 0.04 rad. 0.04
Primary mask Name: Width (mm):	Fixed mask 13 mm 9.00
Primary divergence slit Name: Distance to sample (mm): Type: Height (mm): Angle (°):	Divergence slit 1Â° 50 Fixed 1.52 0.9167
Sample movement type: Rotation time (s):	Spinning 1.0

Diffracted beam path	
Radius (mm):	145.0
Anti-scatter slit Name:	AS slit 9 mm
Type:	Fixed
Height (mm):	9.00
Soller slit Name:	Large Soller slits
Opening (rad.):	0.04 rad.
Filter Name:	0.04
Thickness (mm):	Large beta-filter Nickel
Material:	0.020
	Ni
Detector Name:	PIXcel1D-Medipix3 detector
Type:	RTMS detector
PHD - Lower level (keV):	4.0
PHD - Upper level (keV):	11.3
Mode:	Scanning
Active length (°):	5.542
Source Created by:	Instrument
Application SW:	Data Collector vs. 6.1a
Instrument control SW:	AERIS
Instrument ID:	vs. 1.3.0 20191108 358023
Sample mode	
Reflection	
Scan	
Start time stamp:	05-06-2022 17:34:50
End time stamp:	05-06-2022 17:39:31
Scan axis:	Gonio
Scan range (°):	10.0010 - 79.9999
Start position (°):	10.0024
End position (°):	79.9985
Step size (°):	0.0027
No. of points:	25767
Scan mode:	Continuous
Counting time (s):	1.020

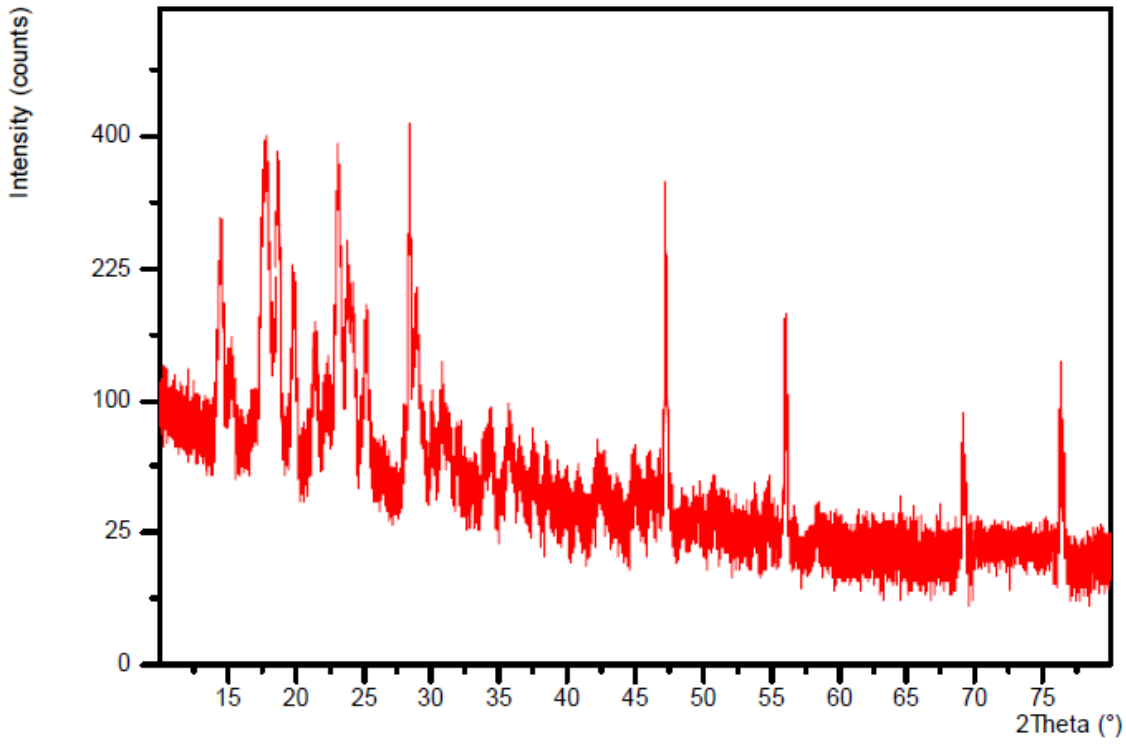


Figure 2: XRD of lamotrigine

U.V spectral analysis:

Determination of λ_{max} of Lamotrigine

The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 6.5 pH phosphate buffer solution prepare suitable dilution to make it to a concentration of 10µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). The spectrum peak point graph of absorbance of Lamotrigine versus wave length was shown in figure 4:

Table 4: Value of λ_{max} in different solvent.

Solvent	**λ max(nm)**
Phosphate buffer P^H 6.5	224 nm

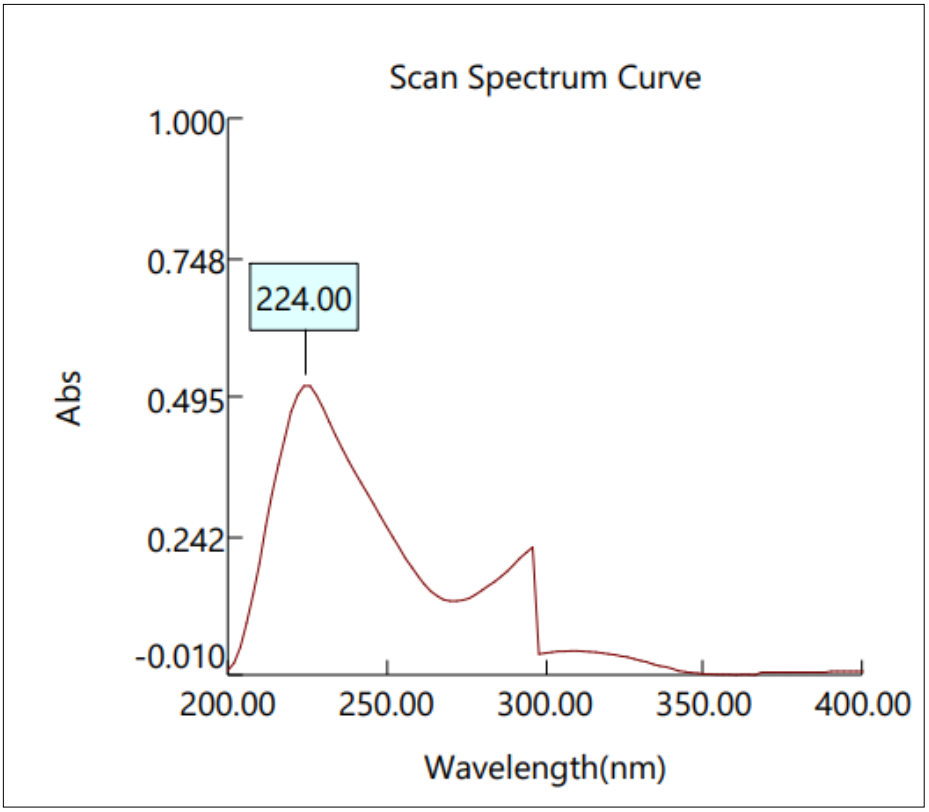


Figure 4 : Determination of λ_{max} of Lamotrigine

Solubility studies:

The solubility of drug in different solvent was given in Table 16. The results revealed good solubility in methanol and poor solubility in NSB.

Table 5: Solubility of lamotrigine in different solvent.

S. No.	Solvents	Solubility (mg/ml) ± SD
1.	NSB	0.386 ± 0.128
2.	NSB with 10% methanol	21.54 ± 0.118
3.	Methanol	65.38 ± 0.086

Determination of Partition Coefficient

The log Partition coefficient (log P) of lamotrigine was found to be 2.334 ± 1.06 (Table 6) which found very closer to reported value.

Table 6: Partition coefficient of Lamotrigine

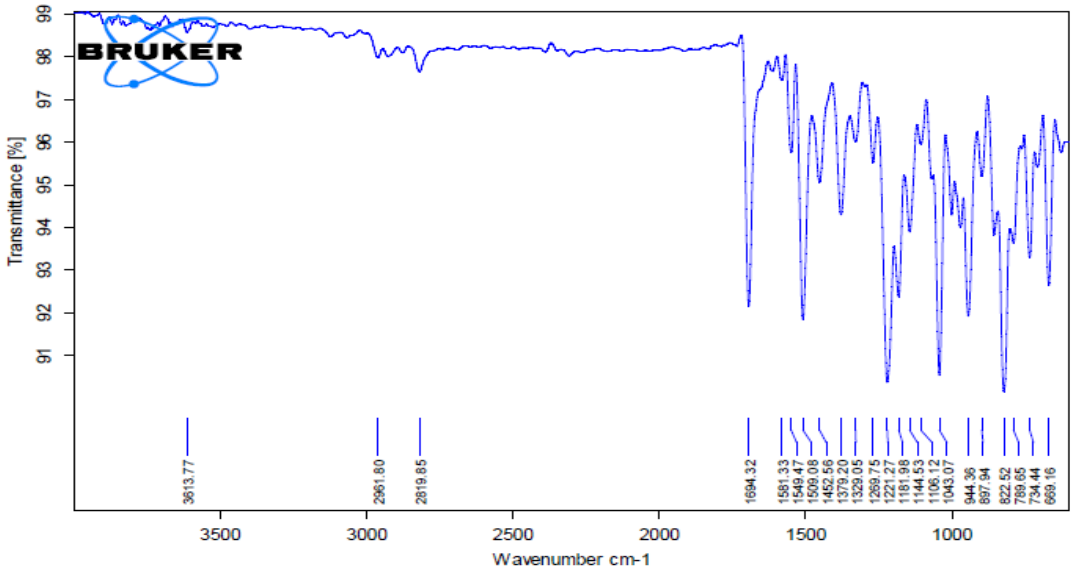
Total amount of drug added (mg)	Amount in organic phase (mg/ml)	Amount in aqueous phase (mg/ml)	Log partition coefficient (± SD)
350	89.69	0.415	2.334 ± 1.06

FTIR study of lamotrigine:

FTIR spectra of lamotrigine shows characteristic peak at different wave number which are elaborated in Table 7. The molecular structure of lamotrigine possessed mainly NH_2 Symmetric, C=N Stretching and C-Cl Stretching. This major characteristic functional groups showed their corresponding infrared absorption peak at mentioned region. The major absorption peaks of IR were found at 3443 cm^{-1} (NH_2), 1546 cm^{-1} (C=N), 748 cm^{-1} (C-Cl). The observed absorption IR peaks were found as per the reported value of IR of lamotrigine .

Table 7: Interpretation of IR spectra of drug lamotrigine

S.No.	IR Peak (cm^{-1})	Interpretation
1.	3443.8	NH_2 Symmetric Stretching
2.	3311	C-H Sym. Stretching
3.	1614	Aromatic breathing + NH_2Scissoring
4.	1546	C-C Stretching+ C=N Stretching+NH_2 Scissoring
5.	1143	Aromatic Breathing + NH_2 Rocking
6.	958	Aromatic Deformation + NH_2 Rocking
7.	748	C-Cl Stretching +C-C-C in plane bending



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Figure 6: FTIR spectra of lamotrigine

HPLC : Retention time (Minutes) 2.072 (Fig. 7)

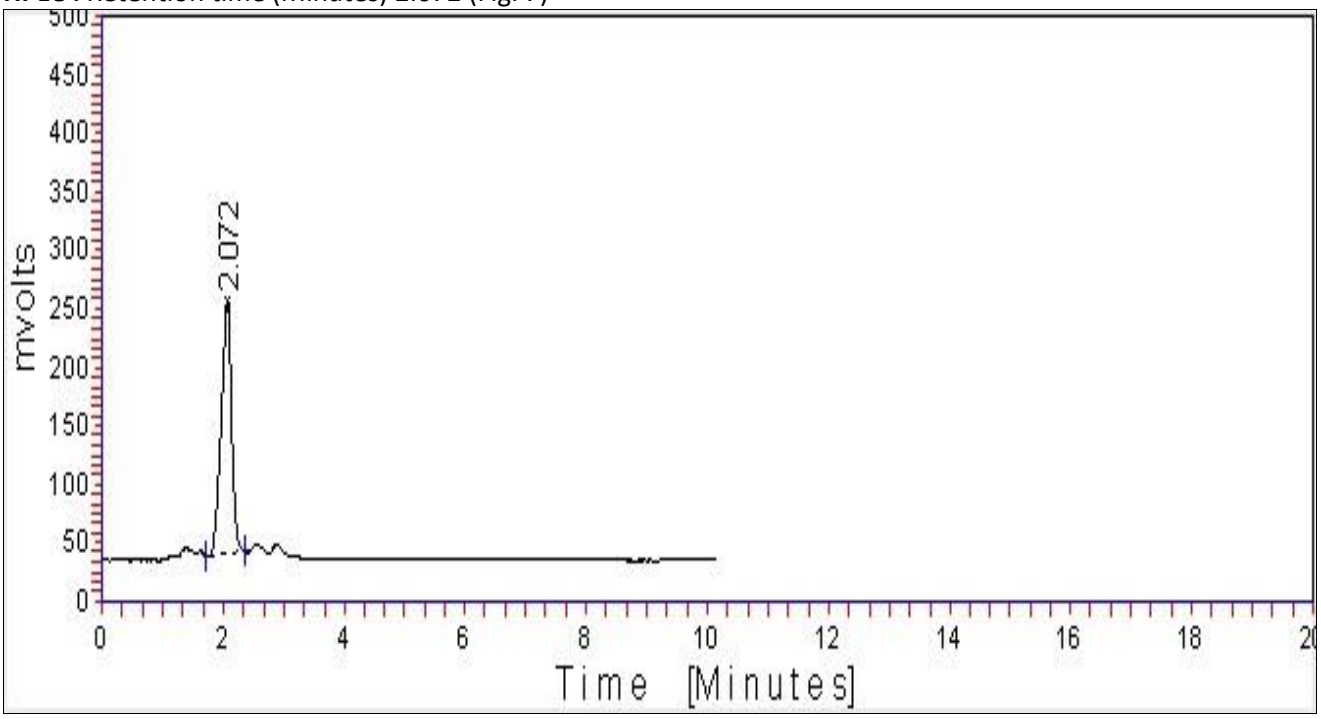


Figure 7: Chromatogram of Lamotrigine

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