Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 1, 2013

Research Article

STABILITY INDICATING ASSAY METHOD FOR QUANTIFICATION OF LACOSAMIDE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM AND CHARACTERIZATION OF MAJOR DEGRADATION PRODUCTS

APEXA PATEL[1], B.N. SUHAGIA[2], ARPIT PATWARI[1]

[1]Department of Pharmaceutical sciences, Saurastra University, Rajkot, [2]Faculty of Pharmacy, Dharamsinh Desai University, Nadiad, Gujarat. Email: apexa178@gmail.com

Received: 29 Oct 2013, Revised and Accepted: 20 Oct 2013

ABSTRACT

Objectives: The present work is aimed to develop sensitive, selective, precise and stability indicating high-performance thin layer chromatographic method for analysis of Lacosamide in both as a bulk and in formulation.

Methods: The method employed TLC aluminum plates precoated with silica gel 60 F_{254} as the stationary phase. The solvent system consisted of toluene: ethyl acetate: methanol: triethylamine (7:2:1:0.1 v/v/v/v). This system was found to give compact spots for Lacosamide (Rf value of 0.30 ±0.02). Lacosamide was subjected to acid and alkali hydrolysis, oxidation, photo degradation and dry heat treatment. Also the degraded products were well separated from the pure drug. Densitometric analysis of Lacosamide was carried out in the absorbance mode at 210 nm. The method was validated for precision, accuracy, ruggedness and recovery.

Results: The linear regression data for the calibration plots showed good linear relationship with r^2 0.994 in the concentration range of 5000-25000 ng. The limits of detection and quantitation were 893.65 and 2708.03 ng per spot, respectively. The drug undergoes degradation under acidic and basic conditions, also in stressed oxidation. All the peaks of degraded product were resolved from the standard drug with significantly different Rf values. Possible impurities were trying to characterization.

Conclusion: The developed high- performance thin layer chromatographic method is sensitive, selective & precise.

Keywords: Lacosamide, HPTLC, Stability Indicating Method, Degradation, Characterization.

INTRODUCTION

Lacosamide is the one of the central nervous system acting drug approved for the treatment of the epilepsy. Lacosamide is a functionalized D-serine derivative in the R- configuration [1]. Lacosamide is chemically [R]-2-acetamido-N-benzyl-3-methoxypropionamide (Figure 1). Lacosamide was approved as an antiepileptic drug for adjunctive therapy of partial onset seizures in the United States and the European union. Lacosamide acts by the mechanism of the enhancement of slow inactivation of voltage gated sodium channel. This inactivation prevents the channel from opening, and helps end the action potential. It is functionalized amino acid. Molecular weight of lacosamide is 250.294 g/mol & molecular formula is $C_{13}H_{18}N_2O_3$ [2-3] It is sparingly soluble in water and slightly soluble in acetonitrile and ethanol [4].Pharmacokinetic parameters for Lacosamide include: rapid and complete absorption from the gastrointestinal tract after oral ingestion (tmax, 1-2 h, bioavailability = 100%); minimal protein binding (less than 30%); linear pharmacokinetic and moderate metabolism to pharmacologically; inactive metabolites (40% excreted unchanged) with subsequent renal excretion and a half-life of 13 h [5-7]. Lacosamide is administered orally as film-coated tablets containing 50, 100, 150 or 200 mg in a syrup containing 15 mg/mL or in a 10 mg/ml solution for intravenous injection [8].

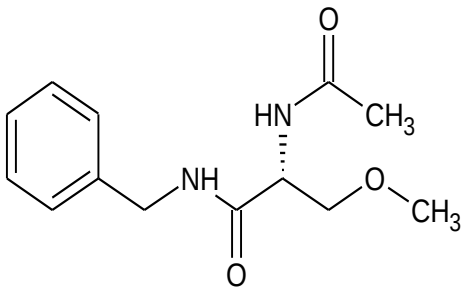


Fig. 1: Chemical structure of Lacosamide

In literature, various spectrophotometric methods and HPLC methods have been reported for quantification of lacosamide in its available market formulation and resolution of impurities and degradation products [9-14].

To our knowledge, no article related to the stability indicating high-performance thin layer chromatographic (HPTLC) determination of lacosamide in pharmaceutical dosage forms has been reported in literature or in Pharmacopoeias. The International Conference on Harmonization (ICH) guideline entitled 'stability testing of new drug substances and products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance [15]. Susceptibility to oxidation is one of the required tests. Also the hydrolytic and the photolytic stability are required. An ideal stability indicating method is one that quantifies the standard drug alone and also resolves its degradation products. A very viable alternative for stability indicating analysis of lacosamide is HPTLC. The advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis. The focus of the present study was to develop an accurate, specific, reproducible and stability indicating method for the determination of low levels of Lacosamide in presence of its degradation products for assessment of purity of the bulk drug and stability of its bulk dosage forms. The proposed method was validated as per ICH guidelines [16].

MATERIALS AND METHODS

Materials

Lacosamide was supplied by Alembic Research Centre, Vadodara India. All chemicals and reagents used were of analytical grade and were purchased from Finar Chemicals, India.

HPTLC instrumentation

The samples were spotted in the form of bands of width 3 mm with a Camag microlitre syringe on precoated silica gel aluminum plate 60 F_{254} (E. Merck, Germany) using a Camag Linomat IV (Switzerland). The mobile phase consisted of toluene: ethyl acetate: methanol: triethylamine (7:2:1:0.1 v/v/v/v). The plates were prewashed by

methanol and activated at 60°C for 5 min prior to chromatography. Samples were applied as bands 3 mm long, at 5 mm intervals under a stream of nitrogen. The slit dimensions were 3 × 0.1 mm. Linear ascending chromatogram development to distance of 8 cm was performed in twin trough TLC developing chamber (Camag) at room temperature and previously saturated for 30 min with mobile phase. Subsequent to the development, TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 210 nm. The source of radiation utilized was deuterium lamp.

Calibration curves

A stock solution of lacosamide (1 mg ml^{-1}) was prepared in methanol. From the resultant solution, 5-25 µL were spotted on the TLC plate to obtain concentrations 5000-25000 ng of lacosamide, respectively. The data of peak area versus drug concentration was treated by linear least square regression analysis and was selected as working range for the assay and recovery. Linearity was also determined over the range of 5000-25000 ng ml^{-1}.

Method validation

a. Linearity

The linearity of response for lacosamide was assessed in the range of 5000-25000 ng per spot for standard drug.

b. Precision

Method Precision (Repeatability)

The precision of the instrument was checked by repeated scanning of the same spot (n=6) of Lacosamide without changing the position of the plate for the HPTLC method.

Intermediate precision (Reproducibility)

The intra and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentration of lacosamide (5000, 15000 and 25000 ng/spot) for the HPTLC method. The results are reported in terms of relative standard deviation.

c. Accuracy

Accuracy of the developed method was accessed by standard addition technique to pre-analyzed sample of market formulation with three concentrations of drug corresponding to 80, 100 and 120% and determining the recovery of added drug. At each level of the amount three determinations were performed.

d. Limit of detection and limit of quantification

The limit of detection (LOD) and quantitation (LOQ) of the drugs were calculated using the following equations as per ICH guidelines.

$$LOD = 3.3 \times \sigma/S \qquad LOQ = 10 \times \sigma/S$$

Where σ is the standard deviation of the response, and S is the standard deviation of y-intercept of regression lines.

e. Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for lacosamide in sample was confirmed by comparing the Rf and spectra of the spot with those of standard. The peak purity of sample was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot.

Assay of marketed formulation

To determine the content of lacosamide in conventional tablets (label claim: 200 mg per tablet), 20 tablets were powdered and powder equivalent to 200 mg of lacosamide was weighed and transferred to 50 mL volumetric flask. 40 mL methanol was added and the flask was sonicated for 15 min. The resultant solution was filtered and volume was made upto the mark with methanol. The resultant solution (10µL) was spotted onto the plate followed by development and scanning. The analysis was repeated in triplicate. The possibility of excipients interference in the analysis was studied.

Accelerated degradation of lacosamide

a. Acid and base induced degradation

Accurately weighed 100 mg of drug was dissolved in 100 ml of methanol. Aliquot (10 mL) was transferred to RBF, 15 mL 1N HCl was added and the solution was refluxed for a period of 30 min. Same procedure was adopted in case of base hydrolysis, wherein concentration of alkali used was 0.05N. The accelerated degradation in acidic and basic media was performed in the dark in order to exclude the possible effects of light on the drug. The resultant solution was neutralized with 1 N NaOH and 0.05 N HCl respectively. The resultant solutions were diluted with methanol upto 50 mL. 10µL of the resultant solution was spotted on TLC plates and plates were developed as stated in 2.2.

b. Hydrogen peroxide induced degradation (Oxidation)

Accurately weighed 100 mg of drug was dissolved in 100 ml of methanol. Aliquot (10 mL) was transferred to RBF and 10 mL 6% H_2O_2 was added to it. This solution was kept at room temperature for 6 hours. The solution was diluted upto 50 mL with methanol. 10µL of the resultant solution was spotted on TLC plates and plates were developed as stated in 2.2.

c. Dry heat degradation

Lacosamide (10mg) was stored at 80 ˚C for 12 hours under dry heat condition. After 12 hours the drug sample was diluted in 50 mL methanol and 10 µL solutions was spotted on TLC plate and plates were developed and analyzed.

d. Aqueous hydrolysis

Lacosamide (10mg) was weighed and transferred to RBF. 30 mL of water was added and the resultant solution was refluxed for 30 min. The solution was diluted with methanol upto 50 mL. The solution (10µL) was spotted on TLC plates and plates was developed and analyzed.

e. Photochemical degradation

The photochemical stability of the drug was also studied by exposing the drug solution to direct sunlight for 48 hours.

RESULTS AND DISCUSSION

HPTLC method development

The TLC procedure was optimized with a view to develop a stability indicating assay method. Both the pure drug and the degraded products were spotted on the TLC plates and run in different solvent systems. The solvent system consisted of toluene: ethyl acetate: methanol: triethylamine (7:2:1:0.1 v/v/v/v) gave good resolution, sharp and symmetrical peak. This system was found to give compact spots for lacosamide (Rf value of 0.30 ±0.02). It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 30 min ensure good reproducibility and peak shape of lacosamide. (Figure II)

Validation

Using the optimized chromatographic conditions, the HPTLC method developed was validated in terms of linearity, LOD, LOQ, precision, accuracy and specificity.

a. Linearity

The linear regression data for the calibration curves (n=3) showed good linear relationship over the concentration range of 5000-25000 ng (co-relation co-efficient 0.994).

b. Precision

Method precision

For lacosamide % relative standard deviation (RSD) was found to be 0.74. The lower value of % RSD indicated that the proposed method is repeatable.

Intermediate precision

For lacosamide % relative standard deviation (RSD) of the intra- and inter-day study was found to be in the range as shown for the HPTLC method (Table 1). The low % RSD values of intra-day and inter-day variations reveal that the proposed method is robust.

c. Accuracy

The recovery experiments were carried out by the standard addition method. The mean percent recovery obtained was 97.72 ± 1.23 (Table 2). The low value of SD indicates that the method is accurate.

d. LOD and LOQ

The LOD and the LOQ for lacosamide was calculated as in the text. LOD and LOQ for lacosamide were found to be 893.65 and 2708.03 respectively. These data show that the method is sensitive for the determination of lacosamide.

Stability in sample solution

Solutions of two different concentrations (5000 and 10000 ng) were prepared from sample solution and stored at room temperature for 2, 4, 6, 10, 12h respectively. They were then applied on the plate, after development the chromatogram was evaluated for additional spots if any. The compound was found to be stable for 6 hours.

Assay of marketed formulation

A single spot at Rf of 0.30 was observed in the chromatogram of the drug samples extracted from conventional tablets. There was no interference from the excipients commonly present in the conventional tablets. The drug content was found to be 99.25 with a % R.S.D. of 1.09. It may therefore, be inferred that degradation of lacosamide had not occurred in the marketed formulation that was analyzed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of lacosamide in pharmaceutical dosage forms.

Accelerated degradation

a. Acid and base induced degradation

The chromatogram of the acid (Figure 3), base (Figure 4) degraded samples shows additional peaks. The concentration of the drug was found to be changing from the initial concentration indicating that lacosamide undergoes degradation under both the conditions. The rate of alkaline degradation was higher as compared to that of acidic condition. In Acid degradation 17.86% degradation & incase of base induced degradation 54.47% degradation occurs.

b. Hydrogen peroxide induced degradation

The sample was degraded in presence of hydrogen peroxide. Additional two peaks are obtained after oxidation. The result indicates lacosamide also degrade under oxidative stress condition. Lacosamide is degraded 73.08% in presence of H_2O_2 (Figure 5)

c. Dry heat degradation

The dry heat sample showed no additional peak. Only 0.20 % drug was degraded on thermal degradation. No significant degradation was observed in standard even after exposure at 80 °C for 12 h. (Figure 6)

d. Aqueous hydrolysis

The Sample was degraded after reflux in presence of aqueous media. 22.18 % lacosamide was degraded in aqueous media. (Figure 7)

e. Photochemical degradation

The drug was not significantly degraded in presence of sunlight (Figure 8)

Characterization

The samples were spotted on TLC plates and the plates were developed. Spots corresponding to major degradation products were scrapped and their mass spectra were recorded. Possible structures of the degradants were proposed on the basis of mass spectra are given below. Mass Spectra of the impurities are given in figure & figure numbers are depicted in Table 1

Table 1:

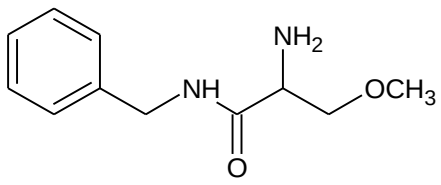
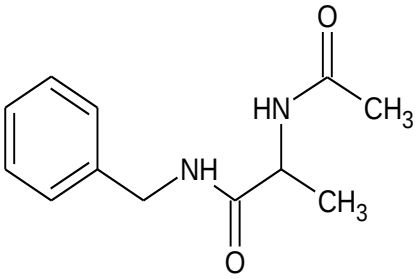
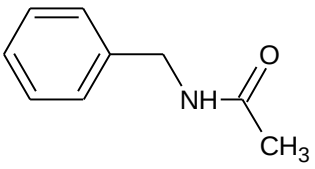
Impurity –A1	Impurity –B1	Impurity- B4
		
Mol wt :209	Mol wt: 219	Mol wt : 149.18
Fig. 9	**Fig. 10**	**Fig.11**

Table 2: Summary of Validation Parameters

Parameter	Data
Linearity range	5000-25000 ng/spot
Correlation coefficient	0.994
LOD	893.65
LOQ	2708.03
Accuracy (n=3)	97.72± 1.23
Precision (%RSD)	
Repeatability	0.75
Intra-day (n=3)	0.48-1.12
Inter-day (n=3)	0.61-1.82

Table 3: Recovery Studies

Amount of standard added to (in ng)	Amount recovered (ng)	Recovery (%)
10000	9975.3	
18000	17697.3	96.52
20000	19875.1	98.99
22000	21693.7	97.65

[a]n=3.

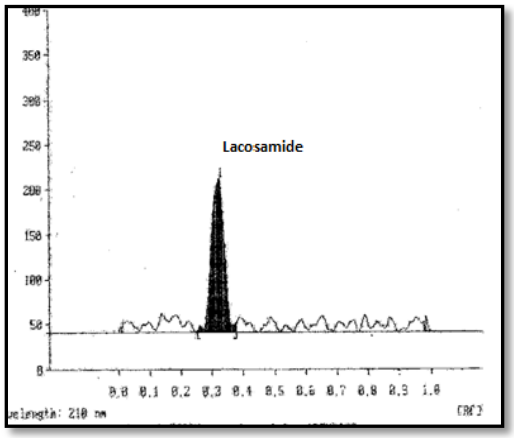


Fig. 2: Chromatogram of standard Lacosamide (R_f 0.30 ±0.02). Mobile phase consisted of toluene: ethyl acetate: methanol: triethylamine (7:2:1:0.1 v/v/v/v)

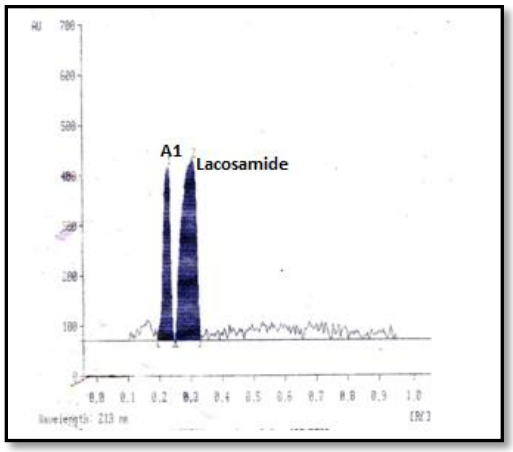


Fig. 3: Chromatogram for acid induced degradation of Lacosamide

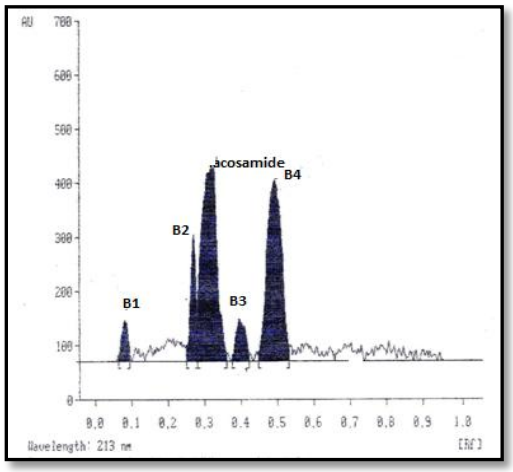


Fig. 4: Chromatogram for base induced degradation of Lacosamide

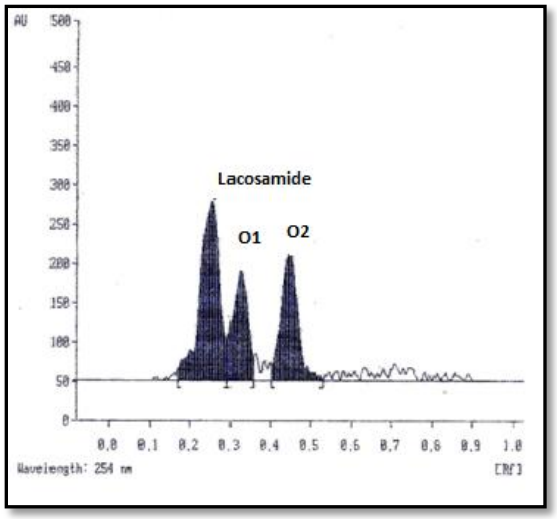


Fig. 5: Chromatogram for Hydrogen peroxide induced degradation of Lacosamide

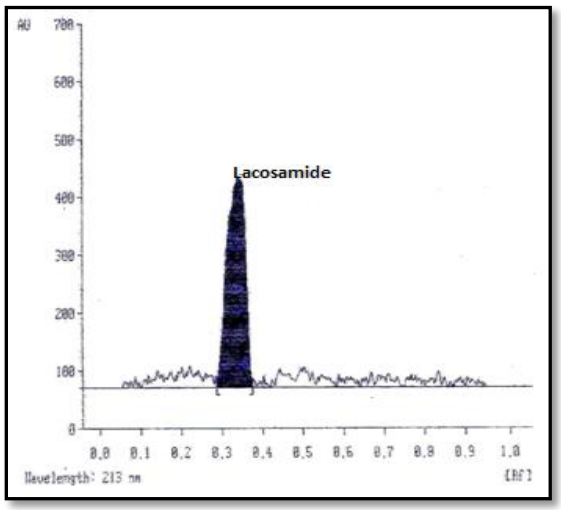


Fig. 6: Chromatogram for Dry heat induced degradation of Lacosamide

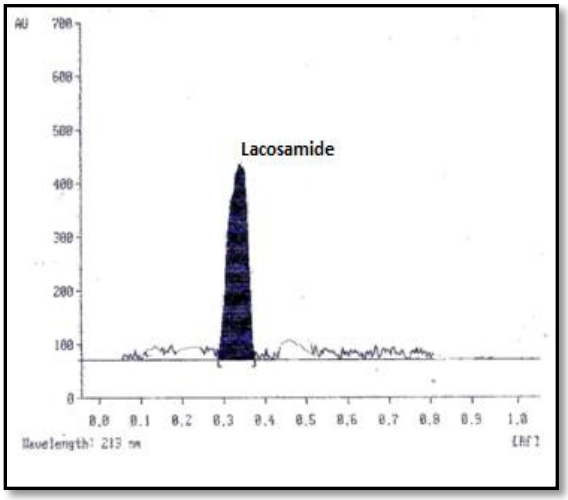


Fig. 7: Chromatogram for Photochemical induced degradation of Lacosamide

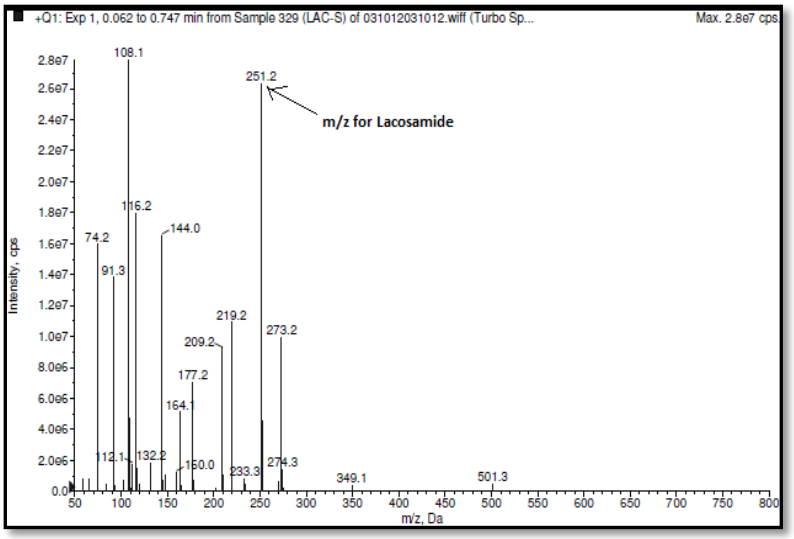


Fig. 8: Mass spectra of Lacosamide

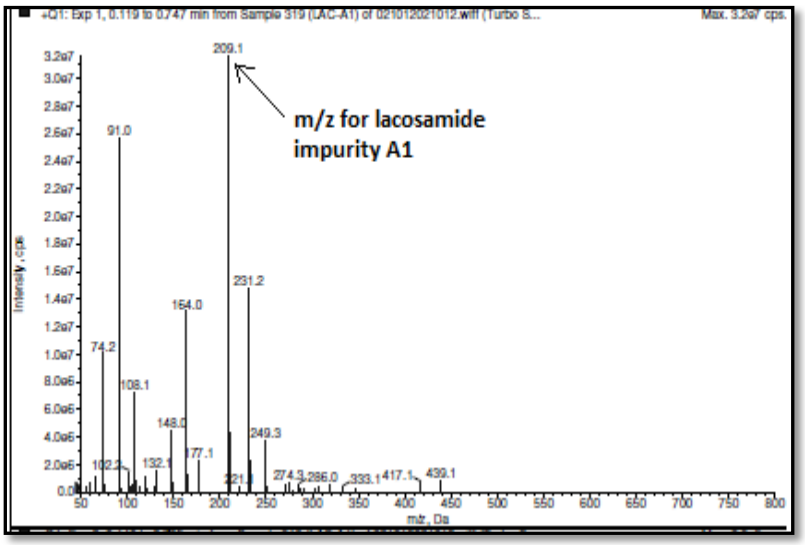


Fig. 9: Mass Spectra of acid induced degradant (A1) of Lacosamide

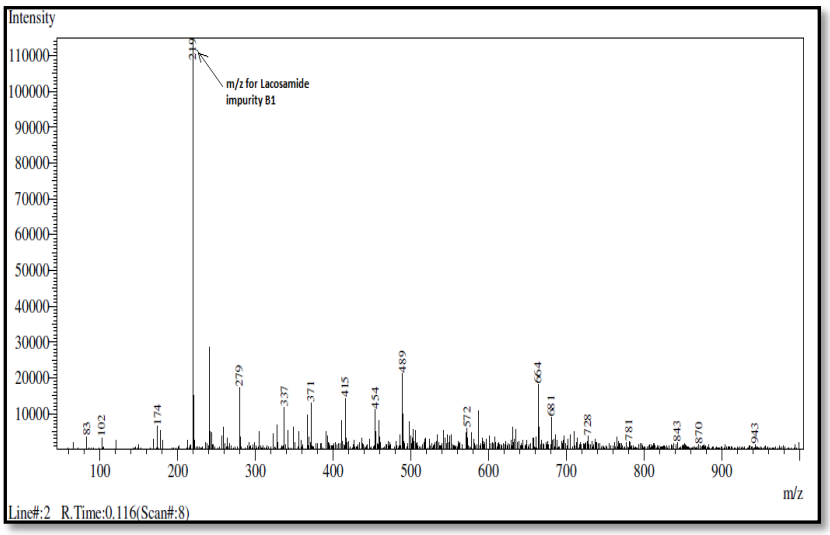


Fig. 10: Mass Spectra of Base induced degradant (B1) of Lacosamide

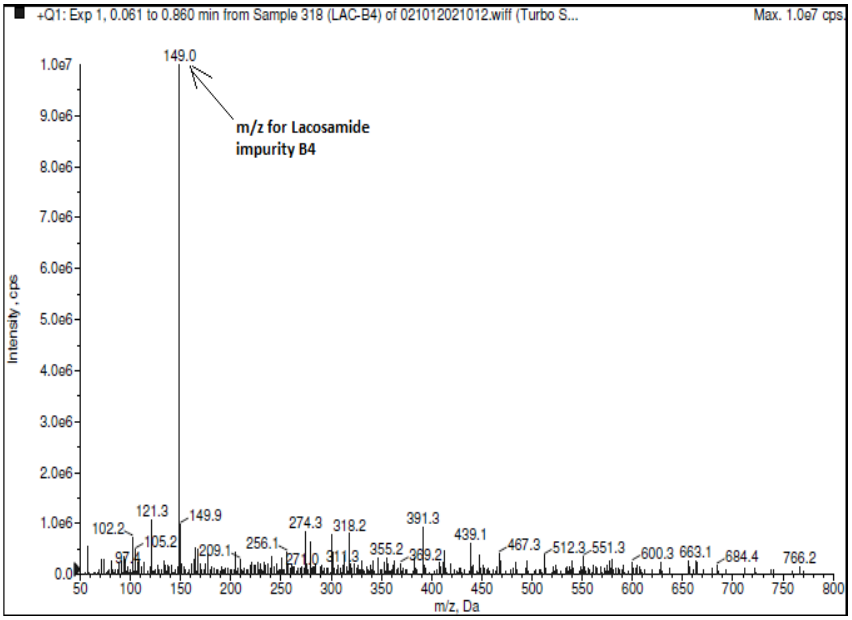


Fig. 11: Mass Spectra of Base induced degradant (B4) of Lacosamide

CONCLUSION

The developed HPTLC technique is precise, specific, accurate and stability indicating one. Statistical analysis proves that the method is reproducible and selective for the analysis of lacosamide as bulk drug and in pharmaceutical formulations. As the method could effectively separate the drugs from their degradation products it can be employed as a stability indicating one.

REFERENCES

1. http://www. Rxlist.com
2. http://www. en.wikipedia.org.
3. Epilepsy currents, © American Epilepsy society 2009; vol 9, No. 5, (September-October) 133-134
4. http://www.epilepsyindia.org/home.asp
5. J. J. Halford. M Lapointe. Epilepsy curr. 2009;1:1-9
6. J.L. Luszczki, Pharmacol Rep. 2009; 61 :197–216
7. E. Ben- Menachem. Drugs Today 2008; 44: 35-40
8. British national formulary. British Medical Association & Royal pharmaceutical society of Great Britain. 58th ed. 2009
9. V. Kalyan Chakravarthy, D. Gowri Shankar; HPLC method for determination of lacosamide S(-)enantiomer in bulk and pharmaceutical Formulation, Rasayan J. Chem 2011; Vol.4, No.4 :744-752
10. Ganji Ramanaiah, D. Ramachandran, G. Srinivas, Srilakshmi, Purnachanda Rao; Development and validation of UV spectroscopy method for estimation of lacosamide in bulk and formulations, Int J Pharm Biomed Sci 2012; 3(1) :10-12
11. K.sai sumanth C. jose gnana babu, s. muneer, M. balaji, P.ulagannathan; Validated spectrophotometric estimation of lacosamide in bulk and tablet dosage form, IJPRD, 2011; vol 3(11),-January(2012) :78-82
12. M.S.Kondawar, S.R.Shinde; Development and Validation of Bioanalytical Method for Lacosamide from Rat Brain Tissues, International Journal of PharmTech Research Oct-Dec 2012; Vol.4, No.4 :1417-1421
13. V.Kalyan Chakravarthy, D.Gowri Sankar; Development and validation of RP-HPLC method for estimation of lacosamide in bulk and its Pharmaceutical formulation; Rasayan. J.chem. 2011;Vol.4, No.3: 666-672
14. R.Valarmathi, S.Farisha Banu, R.Senthamarai, C.S. Dhivya Dhharshini; Simple UV Spectrophotometric method development and Validation for determination of Lacosamide in bulk and its Tablet Dosage Form, International Journal of Drug Development & Research January –March 2013; vol 5, issue 1
15. Stability Testing of New Drug Substance and Products (Q1AR2) ICH Harmonized Tripartite Guidelines.
16. Validation of Analytical Procedure: Methodology (Q2B) ICH Harmonized Tripartite Guidelines.