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Development and validation of stability-indicating assay method for lacosamide by RP- HPLC

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ABSTRACT

The present study describes degradation of Lacosamide under various conditions like, oxidation, hydrolysis, and thermal stress conditions. The drug was found to hydrolyse in acidic and alkaline conditions and no degradation was found in thermal stress condition and oxidation. The separation of the drug and degradation product was successfully achieved on a C_{18} column utilizing water (0.1 % triethylamine and pH 3.0±0.05 was adjusted using Orthophosphoric acid 85%v/v) and methanol in the ratio of 70:30 v/v. The detection wavelength was 215 nm. The method was validated with respect to linearity, precision, accuracy, and specificity. The response was linear in concentration range of 1 - 20 µg/mL. The value of slope and correlation coefficient found to be 42506 and 0.9996 respectively. The R.S.D value for intra- and inter- day precision studies were <1.169 and <1.263, respectively. The recovery of the drug ranged between 98.81 and 101.76% from a mixture of degradation sample.

Introduction

The aims of the present study were to: (1) establish stability of Lacosamide under variety of stress conditions recommended by the International Conference on Harmonization (ICH) [1-2], (2) and develop a stability indicating assay [3].

Lacosamide, (R)-2-acetamido-N-benzyl-3-methoxypropionamide (figure 1) is a functionalized amino acid, in a class of medications called anticonvulsants [4-5]. It works by decreasing abnormal electrical activity in the brain. Lacosamide appears to have a dual mode of action: selective enhancement of sodium channel inactivation and modulation of collapsin response mediator protein-2 [4-5]. Literature was raveled; the literature is silent on stability indicating assay method for Lacosamide under stress conditions. Information on a High-Performance Liquid Chromatography Assay to Monitor the Lacosamide in Patients with Epilepsy is only available [6].

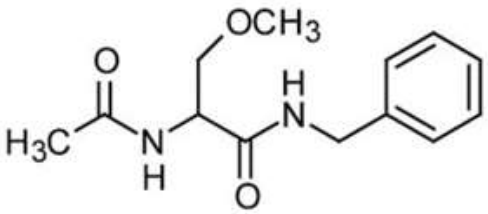

Figure 1: Chemical structures of Lacosamide

Experimental

Materials and chemicals

Lacosamide was received as gift sample from Torrent pharmaceuticals limited (Gujarat, India). Sodium hydroxide and hydrochloric acid were purchased from Merck (Mumbai, Maharashtra). Hydrogen peroxide was procured from S.D. Fine-Chem Ltd. (Boisar, Maharashtra). HPLC grade methanol was purchased from Merck (Darmstadt, Germany). The water for HPLC was prepared by double glass distillation and filtration through Milli-Q water purification system.

Orthophosphoric acid (85% v/v) was obtained from Merck (Darmstadt, Germany).

Instrumentations

Separation studies were carried out using an HPLC consists of Shimadzu LC-10 ADVP solvent delivery system, SIL-10ADVP autoinjector, CTO-10ASVP column oven, SPD M-10AVP photo diode array detector, SCL-10 AVP System controller. . The analytical column used was Lichrosphere C_{18} column (250mm × 4.6 mm i.d, 5 µm particle size). All analysis was carried out at a temperature of 25±2°C under isocratic conditions. All data integration was performed using class VP software. IR spectra were obtained on Infra Red Spectrometer 8300 'Hyper-IR' software - Shimadzu, Kyoto, Japan

Direct mass was taken on gas chromatograph–mass spectrophotometer (QP 5050, Shimadzu, Kyoto, Japan) in chemical ionization (CI) mode.

Degradation studies

Decomposition studies were performed in solutions containing drug at a concentration of 1mg/mL. Samples were withdrawn at suitable time intervals and subjected to HPLC analysis, after suitable dilution. The stress conditions were as follows:

Acid hydrolysis

Solution in 0.1 M hydrochloric acid was kept under reflux at 60±2 ^{0}C and sample was withdrawn at 15min, 30min, 45min, 1h, 1.5h, 2h, 3h, 4h, 6h and 8h respectively and final dilutions were made using mobile phase.

Alkali hydrolysis

Solution in 0.5 M sodium hydroxide was kept at room temperature and sample was withdrawn at 1h, 2h, 4h, 6h 8h, 12h and 24h respectively and final dilutions were made using mobile phase.

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Neutral (water) hydrolysis

Solution in water was kept under reflux at 80 $\pm 2^0$C and sample was withdrawn at 15min, 30min, 45min, 1h, 1.5h, 2h, 3h, 4h, 6h and 8h respectively and final dilutions were made using mobile phase.

Oxidation

Solution in 30% hydrogen peroxide was kept at room temperature and sample was withdrawn at 1h, 2h, 4h, 6h, 8h, 12h, 24h and 48h respectively and final dilutions were made using mobile phase.

Thermal stress

Bulk drug was subjected to dry heat at 70 ± 2^0 C for 7 days.

Isolation and characterization of the major degradation product Solution of Lacosamide in 0.1 M hydrochloric acid (1 mg/mL) was heated at 60 ± 2^0C under reflux condition to allow complete decomposition of drug. The reaction mixture was neutralized by 0.1M sodium hydroxide and freeze dried. Freeze dried product was extracted with ethyl acetate previously treated with anhydrous sodium sulphate. The ethyl acetate extract was completely evaporated on rotary evaporator resulting in a white to off white compound. The compound was subsequently characterized utilizing GC-MS, FTIR.

Separation studies

HPLC studies were carried out first on stressed solutions individually, and then on a mixture of those solutions in which decomposition was observed. Satisfactory separation of the components of the mixture was achieved using a mobile phase composed of water (0.1 % triethylamine and pH 3.0±0.05 was adjusted using Orthophosphoric acid 85%v/v) –methanol in the ratio of 70:30 v/v. The injection volume was 25µL and flow rate was 1 ml/min. The detection wavelength was 215 nm.

Validation of the Method

Linearity and range

The drug solutions were prepared in the concentration range of 1- 20 µg/mL. The solution were injected in triplicate into HPLC column using Milli- Q water (0.1 % v/v triethylamine and pH 3.0±0.05 was adjusted using Orthophosphoric acid 85%v/v)–methanol in the ratio of 70:30 v/v as the mobile phase keeping the injection volume constant (25µL).

Precision

Six injections, of three different concentrations (5, 10, 15 µg/mL), were given on the same day and the values of relative standard deviation were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision.

Accuracy

Accuracy was evaluated by fortifying a mixture of decomposed reaction solutions with three known concentrations (8, 10, 12 µg/mL) of the drug. The recovery of added drug was determined.

Specificity

Specificity was established by determination of purity of the drug peak using a PDA detector. Also, the resolution factor of the drug peak was determined with respect to the nearest resolving peaks.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is ability of analytical method to detect the lowest concentration of the analyte. LOQ is the lowest concentration of the analyte, which can be quantitatively analyzed with acceptable precision and accuracy. It can be calculated based on signal to noise ratio.

Results and Discussion

Development optimization of the stability indicating method

Initial method was developed on pure drug using water (0.1 % triethylamine and pH 3.0±0.05 was adjusted using Orthophosphoric acid 85%) and methanol in the ratio of 65:35 as the mobile phase. The retention time was 7.78 minute. At this ratio of the mobile phase the alkali degradation product mixture was injected but at this ratio of mobile phase drug peak and degradation product peaks were found to be merging so methanol concentration was reduced by 5% as result of which drug peak was resolved at 10.7 minute and resolution could achieved. There was satisfactory resolution.

Validation of the developed stability-indicating method

Linearity

The developed and validated method was linear over the range of 1-20 µg/mL. The coefficient of determination was found to be greater than 0.999±0.002. Typically, the regression equation was found to be Y=42506x - 5714.

Precision

For intra-day precision and inter-day precision, the % relative standard deviation (%RSD) of Lacosamide was found to be <0.926 and <1.263 respectively. These %RSD values were well within the generally acceptable limit of 2%, confirming good precision of the assay method. Result is given in table 1.

Accuracy

The recoveries at three different concentrations were found to be within the range of 98 to 102 % as per ICH guidelines. Mean % recovery (mean ± SD) was found to be 100.34 ± 1.48. The results indicated that the recovery of Lacosamide in three different concentrations was more than 98.81%. Result is given in table 2.

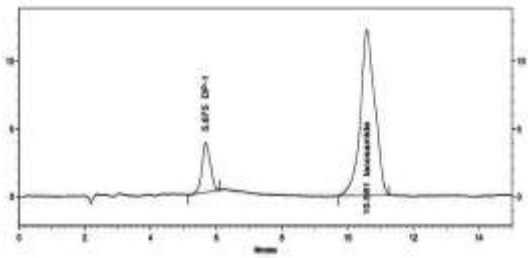
LOD and LOQ

Limit of detection and limit of quantification was found to be 0.0356 and 0.1080 µg mL^{-1} respectively.

Degradation behavior

The following degradation behavior of drug was observed during the above-mentioned HPLC studies:

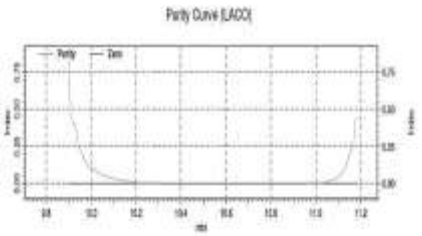
Figure 2: Chromatogram of Lacosamide at 45 min in 0.1 M hydrochloric acid at 60^0 C under reflux condition



Acidic condition

The drug was found to be labile to hydrolysis. On heating the drug solution in 0.1M hydrochloric acid at 60±2°C for 6 hr, 79% degradation was seen with simultaneous rise in degradation product at *R*T 5.6 min.

Figure 3: Peak purity curve of Lacosamide in 0.1M hydrochloric acid at 60 °C under reflux condition



Peak purity index (0.9999) was greater than peak purity threshold (0.9991) which indicate that there is no merging of impurity peaks and peak is pure (figure 2, 3).

Alkali degradation

Lacosamide underwent alkali hydrolysis, but the rate of hydrolysis was higher as compared to that in acid. It took 24 h for the drug to decompose by 68% at room temperature. Peak purity index (0.9999) was greater than peak purity threshold (0.9991) which indicated that there is no merging of impurity peaks and peak is pure (figure 4, 5).

Figure 4: Chromatograph of Lacosamide at 12 hr in 0.5 M sodium hydroxide at room temperature

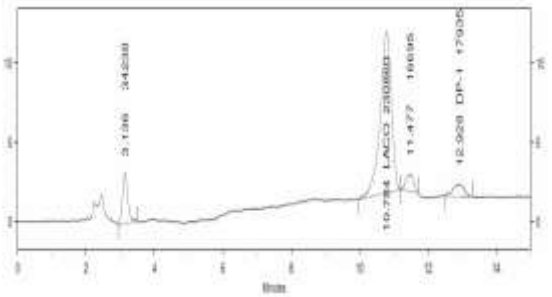
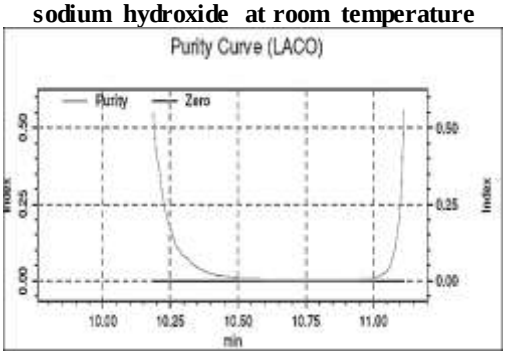


Figure 5: Peak purity curve of Lacosamide at 12 hr in 0.5 M sodium hydroxide at room temperature



Neutral (water) condition

The drug was found to be stable. On heating at 80 ± 2^0C drug was not found to be degraded up to 8 hr

Solid-state study

Studies on solid drug showed that it was stable to the effect of temperature, No decomposition of drug was observed on subjecting it to dry heat at70 ± 2^0C for 7 days.

Oxidative condition

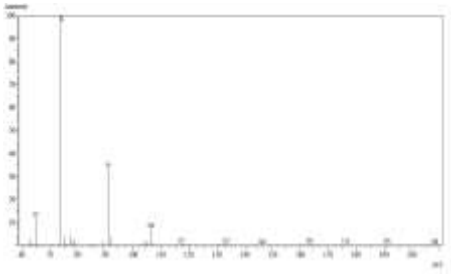
The drug was found to be stable under oxidative condition. No decomposition of drug was observed on subjecting it to oxidative stress condition of 30% H_2O_2 at room temperature for 48 hr.

Identification of major acid degradation compound

The major degradation product characterized to be (*R*)-2-amino-*N*-benzyl-3-methoxypropanamide. Spectral data for characterization is given below.

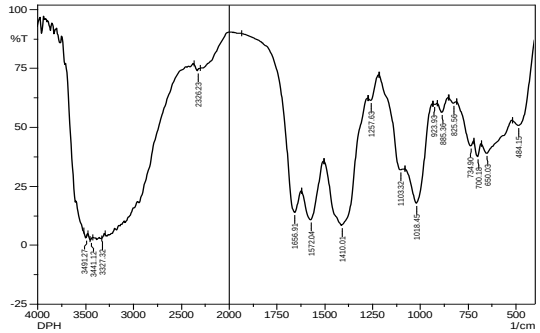
GCMS: Characteristic Peak at retention time of 12.2 min with m/z of 208 (figure 6).

Figure 6: MS spectra of acid degradation compound of Lacosamide



FTIR (cm^{-1}): In Lacosamide characteristic 3288.74 [N-H (2^0)], while in acid degradation product, characteristic 3441 [N-H (NH_2)], 3491.29 (Asymmetric N-H stretching), 1572 (1^0 N-H bending). (Figure 7).

Figure 7: FTIR specrum of acid degradation product of Lacosamide



Conclusion

This study is a typical example of development of a stability-indicating assay, established following the recommendations of ICH guidelines. It is one of the studies where forced decomposition studies were done under all different suggested conditions and the products were resolved in a single isocratic run. The developed method is simple, accurate, precise, and specific. It is proposed for analysis of the drug and degradation products in stability samples in industry.

References

[1] International Conference on Harmonisation. Quality 1A (R2) (2003) Stability Testing of New Drug Substances and Products.

[2] International Conference on Harmonisation. Quality 2(R1) (2005) Note for guidance on validation of analytical procedures: text and methodology

[3] Bakshi M, Singh S. Development of validated stability-indicating assay methods-critical review. J. Pharm. Biomed. Anal. 2002; 28: 1011–40.

[4] European Medicines Agency, Assesssment report for Vimpat. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/000863/WC500050341.pdf (2008) Accessed 10 august 2010.

[5] Emilio P, Uma Y, Gilbert C. peter K. Lacosamide. Nat. Rev. Drug discovery 2008; 7: 973-74.

[6] Ratnaraj N, Greenaway C, Sander JW, Patsalos PN. A High-Performance Liquid Chromatography Assay to Monitor the New Antiepileptic Drug Lacosamide in Patients with Epilepsy. Ther. Drug Monit. 2010; 32: 448 – 52.

Table 1: "Reproducibility and precision data evaluated through intra-day and inter-day studies"

Concentration (µg/mL)	Measured concentration ± S.D.; R.S.D. (%)	
	Intra-day (n=6)	Inter-day (n=6)
5	4.942± 0.0340; 0.688	4.954 ± 0.0533; 1.0763
10	9.863 ± 0.0913; 0.926	9.958 ± 0.1257; 1.2631
15	15.232 ± 0.0306; 0.201	15.397 ±0.1755; 1.1398

Table 2: "Recovery studies"

Concentration (µg/mL)	Measured concentration ± S.D. (µg/mL)	Recovery (%)
8	7.972 ± 0.176	98.81
10	9.870 ± 0.079	101.76
12	12.14 ± 0.024	100.47