International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.4, pp 1417-1421, Oct-Dec 2012

Development and Validation of Bioanalytical Method for Lacosamide from Rat Brain Tissues

M.S.Kondawar, S.R.Shinde*

Department of Quality Assurance, Appasaheb Birnale College of Pharmacy, Sangli. South Shivajinagar, Sangli-Miraj Road, Dist: Sangli, Maharashtra, India. 416416.

***Corres. Author: sushmashinde50@gmail.com**
Mobile No.: 09422513679.

Abstract: Lacosamide is an antiepileptic drug approved in the USA, Europe and several other countries. Lacosamide is currently used to manage partial onset seizures in humans suffering from epilepsy. A rapid, sensitive, novel and simple UV method was developed and validated according to ICH guidelines for the quantification of lacosamide in rat brain homogenate supernatant. Calibration curves were found to be linear ranging from 0.02 to 0.1 µg/ml giving regression of 0.998, using detection wavelength at 215 nm. Precision, limit of detection and limit of quantitation were calculated. Recovery ranged from 97.27 % to 101.45 % w/w. The assay was applied successfully to a pre-clinical study of lacosamide. By applying this method, we were able to determine the brain concentration of lacosamide during at least 1 h after IV administration of 1 mg/kg lacosamide. The method proved to be simple, useful and appropriate, for preclinical and experimental research.

Keywords: Lacosamide; brain tissue; bioanalytical method validation; quantification in rat brain; antiepileptic drug.

1. INTRODUCTION:

Bioanalytical method offers in pharmacokinetic, toxicokinetics, bioequivalence and bioavailability studies at various stages of drug discovery program and support pre clinical and phases I to phase IV clinical trials. [1]
Lacosamide (Figure 1), marketed under the trade name Vimpat, is used for the adjunctive treatment of partial onset seizures and neuropathic pain. [2] Lacosamide has a maximum bioavailability with minimal first-pass metabolism and serum protein binding. Approximately 40% of the drug is ultimately excreted by the kidney with the remainder cleared by metabolism. Lacosamide is functionalized amino acid that acts by selectively enhances slow inactivation of voltage-gated sodium channels and interacts with collapsin response mediator protein-2, a protein mainly expressed in the central nervous system and involved in neuronal differentiation and axonal outgrowth. [3]

There are very few pharmacokinetic data, especially on central nervous system (CNS), reported on lacosamide. Various bioanalytical methods have been published for the determination of lacosamide in biological samples. [4, 5, 6, 7] However, none of these methods included the analysis of lacosamide in brain tissue. The aim of this paper was to establish a simple and sensitive UV procedure for determination of Lacosamide in brain tissue.

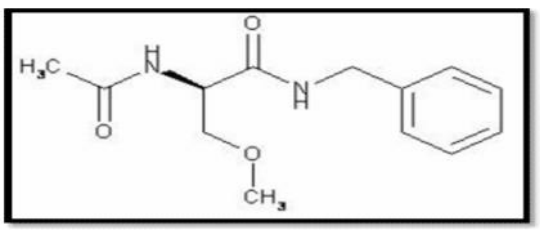

Figure 1: Structure of Lacosamide

2. EXPERIMENTAL

2.1. Apparatus

A UV – Visible double beam spectrophotometer (JASCO), model no. V-550 with 10 mm matched quartz cells was used for experiments operating at 215 nm.

2.2. Reagents and materials

Lacosamide was obtained from Shanku's Chem Sciences PVT. LTD., Baroda. All the reagents and chemicals used were of analytical grade.

2.3. Animals and drug treatments

The UV method was developed for quantification of lacosamide from rat brain after an intravenous administration of lacosamide to male Wistar Albino rats (8 weeks of age, body weight 215–239 g) that were obtained from Appasaheb Birnale College of Pharmacy, Sangli. The experimental studies were performed according to the guidelines of Institutional Ethics Committee (IEC) and followed CPCSEA under Reg. (843/po/ac/04/CPCSEA.27/12/2004). Animals were kept in cages with free access to standard rat diet and water. The animals were maintained at a temperature of 20–26°C with a 12 h light/dark cycle. Before the test, animals were fasted prior to dosing by withholding food overnight, but not water. Lacosamide was dissolved in vehicle dimethly sulfoxide - polyethylene glycol 300-saline (10:27:63, v/v/v) was given as a single intravenous bolus dose via the tail vein (1mg/kg) to rats. The Animals not subjected to any pharmacological treatment were used as a source of drug-free rat brain tissues, which were used as blank matrices in the validation studies. [8]

2.3. Sample preparation

A procedure for the isolation of lacosamide from brain samples prior to UV method developement. Rat brain collection was done after 1h of dosing. Each rat was anaesthetized with ether and sacrificed, decapitated and the whole brain was eased out of the skull. Drug-free (i.e. blank) brain tissue was also obtained from the control rats which were injected with solvent alone. After removal of brain, it was rinsed with cold saline (0.9 % NaCl, g/ml) then surface vasculature ruptured, blotted with dry gauze and weight was taken. The whole brain is homogenized within 1 hour of collection in phosphate buffer (pH 7.4; 0.7 M) (3 ml per rat brain) with a hand-held glass-teflon homogenizer in an ice-cold bath. To induce precipitation of protein, 30 µl of acetonitrile was subsequently added to each brain homogenate and the organic layer (upper layer) was separated by centrifugation and the supernatants was directly used for analysis. [9]

2.4. Preparation of calibration standard solutions

A primary stock solution of lacosamide 1000 µg/ml was prepared by dissolving 10 mg of drug in 10 ml of DMSO .The stock solution of lacosamide was serially diluted with DMSO to achieve working standard solutions of concentrations of 4, 8, 12, 16 and 20 µg/ml. Blank supernatants of the brain tissue homogenates (1 ml) were spiked with the 50 µl of working standard solutions followed by making up volume to 10 ml with DMSO to obtain five calibration standards containing lacosamide having concentrations of 0.02, 0.04, 0.06, 0.08 and 0.1 µg/ml. [10]

2.5. Method Validation

The method was validated according to the existing information, consisting of the study of reliability parameters: linearity, sensitivity, precision, accuracy, recovery, specificity, selectivity.

2.5.1. Selectivity was studied investigating the absence of endogenous interferences from extracts of blank sample supernatant of brain homogenates of rat.

2.5.2. Calibration graphs The homogenate of drug-free brain tissue was spiked with increasing amounts of lacosamide in the concentration range of 0.02–0.1 µg/ml. Calibration graphs were constructed by plotting the absorbance of the drug against the concentration.

2.5.3. The linearity was determined at different concentrations. For inter-day, assay was performed over 5-days separately, while the intra-day assay was performed for 1 day by analysing each concentration 3 times. Calibration curves were plotted as absorbance against the concentration. From this curve the lower limit of quantitation was determined.

2.5.4. Precision To determine intraday precision, three replicate analysis of samples were performed on the same day. The interday precision was accessed by analysis of samples on five different days. Precision was expressed as % CV.

2.5.5. Accuracy was determined by performing recovery studies by spiking different concentrations of lacosamide in the preanalyzed sample. All analysis was performed in triplicate.

Percentage drug recovery for analyte with corresponding %CV was determined. [11]

2.5.6. The limit of detection (LOD) and the limit of quantification (LOQ) were determined using following equations

LOD =3.3 σ / s **LOQ= 10 σ / s**

Where LOD and LOQ expressed in concentration, S is the slope of the calibration plot, and σ the standard deviation of the y- intercept. [12]

2.5.6. Application of method The amount of lacosamide in brain was calculated as,

$$\frac{(V \times C)}{M} \text{ (ng/g of brain tissue)}$$

Where,

V represents the total volume of the reconstituted extract (ml),

C represents the concentration of the reconstituted extract determined by UV (ng/ml) and

M represents the weight of rat brain (g). [13]

3. RESULT AND DISCUSSION

The proposed method for determination of brain concentrations of lacosamide is based on UV-spectrophotometer. Lacosamide dissolve much better in DMSO, which is used as solvent for spectrophotometric method. The method was validated as per the ICH guidelines

3.1. Linearity and sensitivity Lacosamide was showed linearity in the concentration range of 0.02-0.1 µg/ml with correlation coefficient of 0.998. Endogenous material from homogenised brain did not impact on the quantification of Lacosamide. Calibration curves were obtained over the concentration range of 0.02–0.1µg/ml. The inter-day linear regression equation of the brain homogenate was (Y= [1.290] X− [0.002], R^2=0.997 and for the intra-day it was (Y= [1.203] X− [0.002], R^2=0.998.

3.2. Limits of quantitation the lowest calibration standard corresponded to the LOQ were found at 0.004 µg/ml and for LOD at 0.001 µg/ml for lacosamide in supernatant of tissue homogenate.

3.3. Precision The result obtained for intra-day and inter-day precision are shown in table 1 and 2 respectively. The Result of analysis showed satisfactory values of ± S.D, % CV and % relative standard deviation which indicates that method is precise and reproducible (Table 1 and 2).

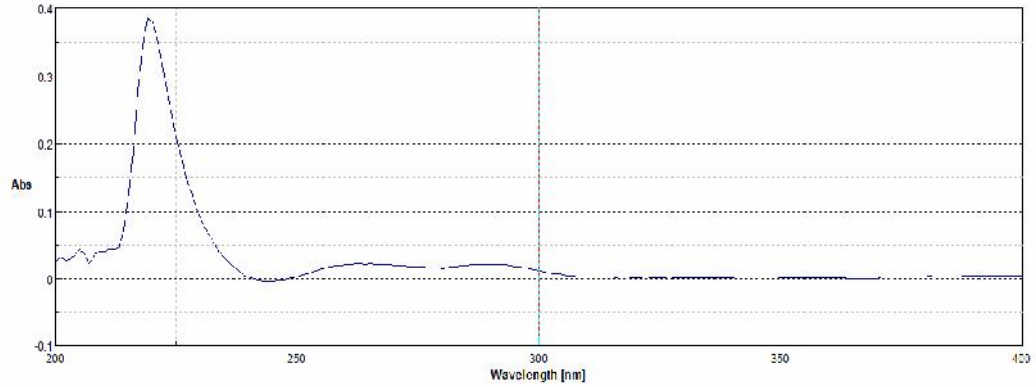

Fig. No.2 : UV Spectra of Lacosamide

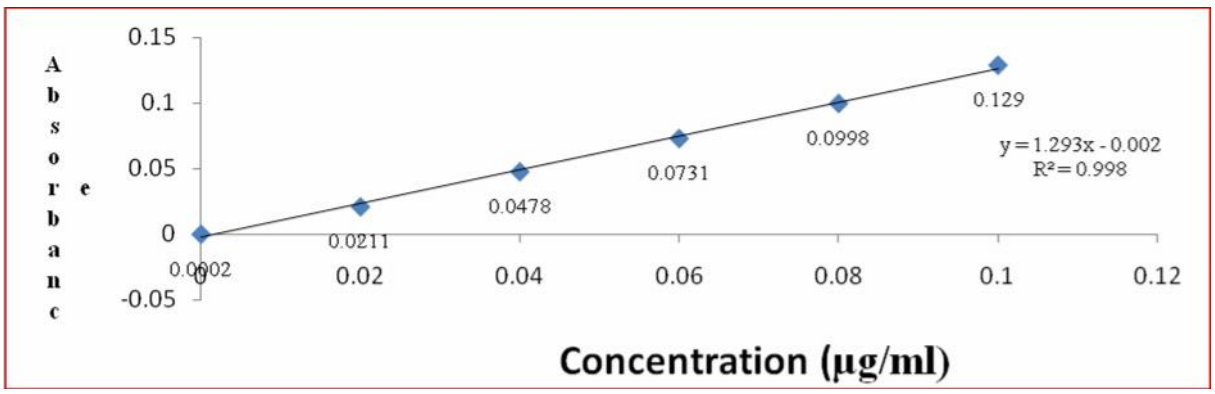

Fig. No. 7: Calibration curve of Lacosamide

Table .1: Intra-day variability of lacosamide

Nominal Conc. (µg/ml)	Mean observed conc. (µg/ml)	% Conc. found (Mean ± SD)	RSD (%)	Coefficient Of variance (%)
0.02	0.02	100 ± 0.0003	1.70	1.1074
0.04	0.0387	96.75 ± 0.0003	0.07	0.7751
0.06	0.0582	97 ± 0.0003	0.14	1.03
0.08	0.0789	98.62 ± 0.0005	0.20	0.253
0.1	0.1000	100 ± 0.0005	0.15	0.003

Table .2: Inter-day variability of Lacosamide

Nominal Conc. (µg/ml)	Mean observed conc. (µg/ml)	% Conc. found (Mean ± SD)	RSD (%)	Coefficient Of variance (%)
0.02	0.02	100 ± 0.0003	1.68	1.66
0.04	0.0386	96.75 ± 0.0003	0.62	0.259
0.06	0.0581	97 ± 0.0003	0.5	0.344
0.08	0.0791	98.62 ± 0.0005	1	0.505
0.1	0.1000	100 ± 0.0005	0.31	0.983

Table.3: Accuracy of the method for the determination of lacosamide in rat brain.

Recovery (%) of lacosamide in spiked rat brain					
Recovery level (%)	Initial concentration (µg/ml)	Spiked concentration (µg/ml)	Recovery (%)	RSD (%)	Coefficient Of variance (%)
Intra day					
80	0.05	0.04	98.55 %	0.78	0.801
100	0.05	0.05	101.45 %	1.16	0.895
120	0.05	0.06	97.27 %	1.13	1.70
Inter day					
80	0.05	0.04	96.44 %	1.18	0.4028
100	0.05	0.05	98.31 %	0.84	0.895
120	0.05	0.06	98.95 %	0.69	0.843

3.4. Accuracy

The values of standard deviation were satisfactorily low and recovery was found to be in the range of 96.44-101.45 % which indicates reproducibility and accuracy of this method (Table 3). This method has been shown to be suitable for the determination of the concentrations of lacosamide after 1 hr of IV administration of lacosamide to rat, the brain concentration was found to be 366.66 ng/g.

4. CONCLUSION:

All the above results indicate that, the proposed spectrophotometric method is very simple, accurate, rapid, precise, and sensitive for determination of lacosamide concentration in rat brain. The developed method provides calibration curve linear ranging from 0.02-0.1 µg/ml. The % RSD for all parameters was found to be less than two, which revealed the validation of new method and results obtained are in acceptance limits. LOD and LOQ indicate that very small quantities of drug can be estimated by this method. Method facilitates quantitative recovery of lacosamide from the brain matrix without interference from the major metabolites of lacosamide and brain endogenous matter with excellent recovery ranging from 96.44-101.45 %. The method was found to yield better results and it is applicable to *in vivo* evaluation of the concentration of lacosamide in rat brain.

4. ACKNOWLEDGEMENT:

Prof. D.D. Chougule, Principal of Appasaheb Birnale College of Pharmacy, Sangli, is acknowledged for providing all kind of facilities and his valuable support for this work. Authors are thankful to Shanku's Chem Sciences PVT. LTD., Baroda for providing the free gift sample of lacosamide and to Prof. S. B. Patil for their kind support.

5. REFERENCES:

1. Pandey S., Pandey P., Tiwari G., Tiwari R., Bioanalysis in drug discovery and development, Pharmaceutical methods, 2010, 1 (1), 14-24.
2. Bettina K. Beyreuther, Freitag J, Heers C, Krebsfanger N, Scharfenecker and Thomas Stohr, Lacosamide: A review of preclinical properties, CNS drug reviews, 2007, 13 (1), 21-42.
3. Krasowski M. D., Therapeutic Drug Monitoring of the Newer Anti-Epilepsy Medications, Pharmaceuticals (Basel),S 2010, 3 (6), 1909–1935.
4. Greenaway, Ratnaraj C., Sander N., Patsalos J. W., Saliva and serum lacosamide concentrations in patients with epilepsy, EPILEPSIA, 2011, 52 (2), 258 – 263.
5. Beyreuther BK, Geis C, Stohr T, Sommer C, Antihyperalgesic efficacy of lacosamide in a rat model for muscle pain induced by TNF, Neuropharmacology, 2007-04, 52(5), 1312-7.
6. Kestelyn C., Lastelle M., Higuet N., Dell'Aiera S., Staelens L., Boulanger P., Boekens H.Smith S., A simple HPLC–UV method for the determination of lacosamide in human plasma, Bioanalysis, November 2011, 3 (22), 2515-2522.
7. Greenaway, Ratnaraj C., Sander N., Patsalos J. W., A High-Performance Liquid Chromato graphy Assay to Monitor the New Antiepileptic Drug Lacosamide in Patients with Epilepsy, Therapeutic Drug Monitoring, 2010, 32 (4), 448-452.
8. Ichikawa N., Naora K., Hirano H., Iwamoto K., Quantitation of acetazolamide in rat plasma, brain tissue and cerebrospinal fluid by high-performance liquid chromatography, Journal of pharmaceutical and biomedical analysis, 1998, 17, 1415-1421.
9. Kim S. J., Koo T. S., Jin D., Baek M, Lee S. K., Shin D. S., Moon H., Liquid chromatography-tandem mass spectrometry for quantification of lacosamide, an antiepileptic drug, in rat plasma and its application to pharmacokinetic study, Biomed. Chromatography, 2011.
10. Alvesa G, Figueiredoa l, castel-Brancoa M, Loureirob A, Falcaoa A, and Caramonaa M, Simultaneous and enantioselective liquid chromatographic determination of eslicarbazepine acetate, S-licarbazepine, R-licarbazepine and oxcarbazepine in mouse tissue samples using ultraviolet detection, Analytica Chimica Acta, 16July2007, 596 (1), 132-140.
11. Braggio S., Barnaby R. J., Grossi P., Cugola M., A Strategy for validation of bioanalytical methods, Journal of Pharmaceutical and Biomedical Analysis, 1996, 14, 375-388.
12. Castel-Branco M. M., Almeida A. M., Falcao A. C., Macedo T. A., Caramona M. M., Lopez F. G., Lamotrigine analysis in blood and brain by high-performance liquid Chromatography, Journal of Chromatography B, 2001, 755 119–127.
13. Owen A., Tette J. N., Morgan P., Pirmohamed M., Park B. K., LC determination of carbamazepine in murine brain, Journal of Pharmaceutical and Biomedical Analysis, Nov.2001, 26(4), 573-577.

*****