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METHOD DEVELOPMENT AND VALIDATION OF LACOSAMIDE DRUG BY USING RP-HPLC

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ABSTRACT

A stable, simple, rapid, precise, accurate HPLC method for analysis of Lacosamide was developed and validated as per ICH guidelines without need of any internal standard. Separation was carried out using ODSColumn$_{18}$ (250mm x 4.6 mm i.d.), 5µ with Water : Acetonitrile (50:50v/v) as a mobile phase with flow rate 1.0ml/min and the column temperature was Ambient. U.V Detection was performed at 215nm. The parameters studied were retention time, linearity and range, accuracy, precision.

INTRODUCTION

Lacosamide tablets are indicated as adjunctive therapy in the treatment of partial –onset seizures in patients with epilepsy aged 17 years and older. Chemical name of Lacosamide, the single (R)-enantiomer, is (R) - 2-acitamido – N-benzyl-3methoxy propionamide (IUPAC). Lacosamide is a functionalized amino acid. Its molecular formula is $C_{13}H_{18}N_2O_3$, and its molecular weight is 250.30. Lacosamide is a white to light yellow powder. It is sparingly soluble in water and slightly soluble in Acetonitrile and ethanol [1].

It is not official in any pharmacopoeia, few liquid chromatography procedures have been reported for the determination of Lacosamide. The author has developed a liquid chromatographic method which would serve as a rapid and reliable method for the determination of Lacosamide in Bulk and pharmaceutical dosage forms [2].

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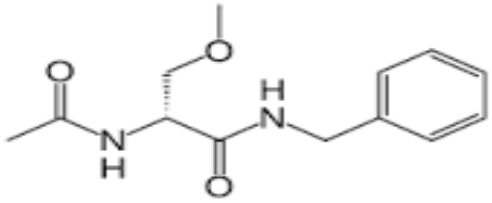
Structure of Lacosamide



MATERIALS AND METHODS

Water : HPLC Grade (Millipore),
Acetonitrile: HPLC Grade.

The linearity of the response of drug was verified from 4µg/ml-24µg/ml concentrations. The calibration graphs were obtained by plotting the responses versus concentrations.

Preparation of mobile phase

The separation was carried out under isocratic elution with mobile phase was a mixture of Water : Acetonitrile (50:50v/v)

Chromatographic conditions

Column : C_{18} column (250mm x 4.6mm)
Flow rate : 1.0ml/min
Wave length : 215nm
Injection volume : 20µl

Standard Preparation: (For Lacosamide Tablet – 100mg)

Accurately weigh and transfer of about 20.0mg of Lacosamide working standard into a 100 ml volumetric flask, add 60 ml of diluents and sonicate to dissolve. Cool the solution at room temperature and dilute to volume with diluent. Transfer 2.0 ml of above solution into 50 ml volumetric flask and to dilute to volume with diluents [3].

Sample preparation: (For Lacosamide Tablet – 100mg)

Weigh and finely powder not fewer than 10 tablets. Accurately weigh and transfer equivalent to 100mg of Lacosamide in 250ml volumetric flask add about 100 ml of diluent , shake for 10 minutes on orbital shaker and sonicate for 30 minutes with occasional shakings cool the solution to room temperature and dilute to volume with diluent and mix. Filter the solution through 0.45µm membrane filter. Transfer 2.0ml of the above solution into a 25ml volumetric flask and dilute to volume with diluents [4].

System Precision

Standard solution were injected into HPLC system and the area of the peak and RSD was calculated.

Accuracy

Accuracy of the developed method was studied by recovery experiments. The same solutions were analyzed for percentage recovery studies at three levels for each formulation. The assay results were expressed as percentage of label claim of amount of Lacosamide found in the tablet formulations.

These solutions were analysed for its percentage drug contents with respect to label claim, by a single drug [5].

RESULTS

To develop a suitable and LC method for determination of Lacosamide, different mobile phase were employed to achieve the best separation and resolution. The method development was started with ODS (250mmx4.6mm id) 5µ, with the following mobile phase. Accurately weigh and transfer about 1.37grams of sodium di-hydrogen phosphate monohydrate in 1000ml of purified water and mix. Adjust the pH to 3.0 with dilute orthophosphoric acid solution. Filter through 0.45µm membrane filter. Prepare a filtered and degassed mixture of Buffer and Aceonitrile in the ratio of 800:200v/v respectively, peaks obtained were in the form of Tailing . For the next trail the mobile phase composition was changed with Water in place of Buffer.

The mobile phase composition Changed respectively Water and Acetonitrile in the ratio of 50:50v/v respectively as eluent at flow rate 1.0ml/min and the column temperature was Ambient. U.V detection was performed at 215nm. The retention time of Lacosamide comes at the 3.378 mins, Which is Within acceptance criteria according to ICH guidelines, so this was accepted.

Table 1. For Optimization of chromatographic conditions

S.no	Parameters	Trail-1	Trail-2	Trail-3	Trail-4
1.	Column	ODS, C_{18} (250mmx4.6mm), 5µ	ODS, C_{18} (250mmx4.6mm), 5µ	ODS, C_{18} (250mmx4.6mm), 5µ	ODS, C_{18} (250mmx4.6mm), 5µ
2.	Injection volume	20µ1	20µ1	20µ1	20µ1
3.	Run time	3min	3min	3min	3min
4.	Mode	isocratic	Isocratic	Isocratic	isocratic
5.	Flow rate	0.5 ml/min	1.2ml/min	1.4ml/min	1.6ml/min
6.	Retention time	3.396min	1.927min	1.903min	1.354min
7.	Wave length	215nm	215nm	215nm	215nm
8.	Mobile phase	ACN:H_2O	ACN:H_2O	ACN:H_2O	ACN:H_2O

Table 2a. For Linearity

S.no	Parameters	Trail-1	Trail-2	Trail-3	Trail-4
1.	Column	ODS,C_{18} (250mmx4.6mm), 5µ	ODS,C_{18} (250mmx4.6mm), 5µ	ODS,C_{18} (250mmx4.6mm), 5µ	ODS,C_{18} (250mmx4.6mm), 5µ
2.	Injection volume	20µ1	20µ1	20µ1	20µ1
3.	Run time	3min	3min	3min	3min
4.	Mode	Isocratic	Isocratic	Isocratic	isocratic
5.	Concentrations	0.1µg/ml	0.2µg/ml	0.3µg/ml	0.4µg/ml
6.	Retention time	3.007min	3.009min	3.015min	3.017min
7.	Wave length	215nm	215nm	215nm	215nm
8.	Mobile phase	ACN:H_2O	ACN:H_2O	ACN:H_2O	ACN:H_2O

Table 2b. For Linearity

S.no	Parameters	Trail-5	Trail-6	Trail-7
1.	Column	ODS, C_{18} (250mmx4.6mm), 5µ	ODS, C_{18} (250mmx4.6mm), 5µ	ODS, C_{18} (250mmx4.6mm), 5µ
2.	Injection volume	20µ1	20µ1	20µ1
3.	Run time	3min	3min	3min
4.	Mode	Isocratic	Isocratic	Isocratic
5.	Concentrations	0.5 ml/min	0.6ml/min	0.7ml/min
6.	Retention time	3.023min	3.363min	3.654min
7.	Wave length	215nm	215nm	215nm
8.	Mobile phase	ACN:H_2O	ACN:H_2O	ACN:H_2O

Table 3a. For Accuracy

S.no	Parameters	Trail-1	Trail-2	Trail-3	Trail-4
1.	Column	ODS, C_{18} (250mmx4.6mm), 5µ	ODS, C_{18} (250mmx4.6mm), 5µ	ODS, C_{18} (250mmx4.6mm), 5µ	ODS, C_{18} (250mmx4.6mm), 5µ
2.	Injection volume	20µ1	20µ1	20µ1	20µ1
3.	Run time	3min	3min	3min	3min
4.	Mode	Isocratic	Isocratic	isocratic	isocratic
5.	Concentrations	80ppm	80ppm	80ppm	100ppm
6.	Retention time	3.41min	3.41min	3.41min	3.41min
7.	Wave length	215nm	215nm	215nm	215nm
8.	Mobile phase	ACN:H_2O	ACN:H_2O	ACN:H_2O	ACN:H_2O

Table 3b. For Accuracy

S.no	Parameters	Trail-5	Trail-6	Trail-7	Trail-8
1.	Column	ODS, C_{18} (250mmx4.6mm), 5µ	ODS, C_{18} (250mmx4.6mm), 5µ	ODS, C_{18} (250mmx4.6mm), 5µ	ODS, C_{18} (250mmx4.6mm), 5µ
2.	Injection volume	20µ1	20µ1	20µ1	20µ1
3.	Run time	3min	3min	3min	3min
4.	Mode	isocratic	Isocratic	isocratic	Isocratic
5.	Concentrations	100ppm	100ppm	120ppm	120ppm
6.	Retention time	3.37min	3.37min	3.37min	3.37min
7.	Wave length	215nm	215nm	215nm	215nm
8.	Mobile phase	ACN:H_2O	ACN:H_2O	ACN:H_2O	ACN:H_2O

Table 4. Precision readings

HPLC Injection Replicates of Lacosamide	Retention time	Area
Replicate -1	2.78	1.786
Replicate-2	2.78	1.772
Replicate-3	2.78	1.796
Replicate-4	2.75	1.572
Replicate-5	2.75	1.552
Replicate-6	2.75	1.514
Average	2.76	1.665
Standard deviation	0.0364307562	1.3199549348
%RSD	0.1710513794	9.2721222316

Table 6. Linearity Results

Concentration	Area
0	0
4	8.6
8	18.5
12	28.2
16	38.5
20	48.6

Fig 1. Linearity curve

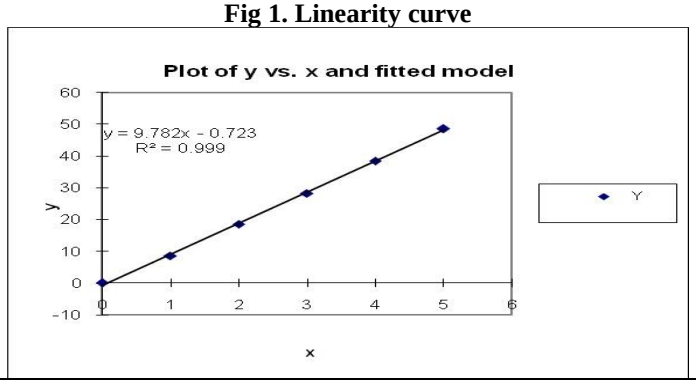
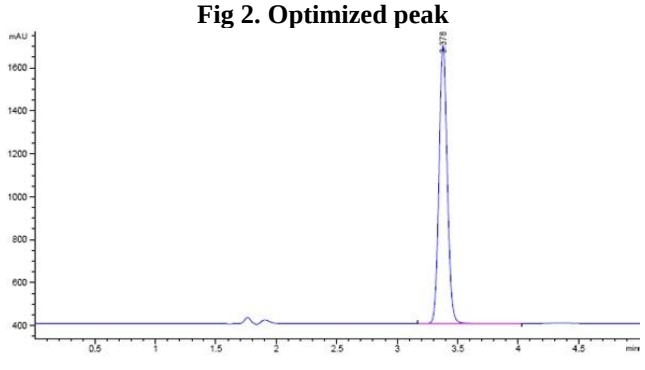


Fig 2. Optimized peak



CONCLUSION

The proposed method was found to be simple, sensitive, rapid and economical for the determination of Lacosamide. The method was found to be linear, precise and accurate. The mean percentage recovery above 98.99% indicates the reproducibility and accuracy. The simple recoveries in all formulations were in good agreement with their respective label claim and they suggest non-interference of formulation recipients in the estimation. After validating proposed methods as per ICH guidelines and correlating obtained values with the standard values, satisfactory results were obtained.

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