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Quantitative estimation of related compounds of lacosamide in oral solution by using reverse phase HPLC

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ABSTRACT

A novel gradient reverse phase HPLC method has been developed for quantitative estimation of Lacosamide impurities in pharmaceutical dosage form. Chromatographic separation was achieved on Waters Symmetry C8, 250 x 4.6mm, 5µ. Detection wavelength was set at 210 nm. Drug product was subjected for stress conditions of Acid, Alkali and Peroxide degradation. Lacosamide was found to be degrading significantly in Acid and alkali conditions. Peak purity of Lacosamide was found to be passing, indicates that all degradents are separated from the analyte peaks. The developed method was validated as per ICH guidelines with respect to Specificity, Linearity, Accuracy, Precision, Limit of quantification, Robustness and solution stability.

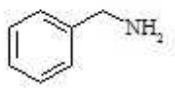
Key words: Lacosamide, oral solution, Impurities, and Validated HPLC.

INTRODUCTION

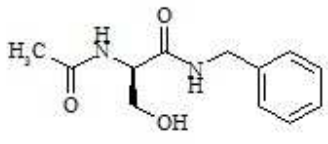
Lacosamide is the one of the central nervous system acting drug approved for the treatment of the epilepsy. Lacosamide is a functionalized D-serine derivative in the R- configuration [6]. Lacosamide is chemically [R]-2-acetamido-N-benzyl-3- methoxypropionamide.

A literature survey reveals that several methods have been reported for determination of assay of Lacosamide. Few methods have been reported for the estimation of impurities of Lacosamide [1-11]. However, there are no methods found to determine all the probable impurities of Lacosamide in oral solution product. Hence attempt was made to develop and validate a HPLC method for the estimation of impurities in Lacosamide oral solution.

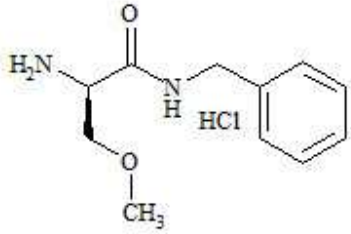
For this study Benzyl amine, DAM Impurity, Related compound-02, Related compound-03, Related compound-04, and Related compound-05 [Fig 1] of Lacosamide has been considered. Forced degradation studies in Acid, Alkali and Peroxide conditions by using PDA detector were performed to ensure that degradents were separated from analyte peak.



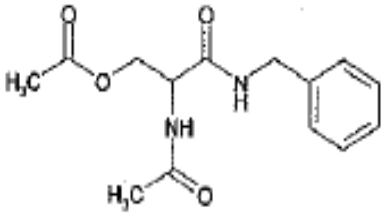
Benzylamine



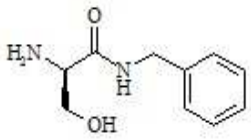
Related compound -05



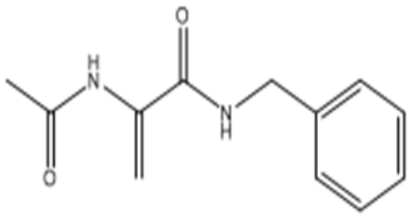
Related compound -02



Related compound -03



DAM impurity



Related compound -04

Fig 1. Chemical structure of Lacosamide impurities

MATERIALS AND METHODS

2.1 Materials : Chemicals and reagents:

Potassium dihydrogen phosphate, Di sodium hydrogen phosphate anhydrous, Ortho phosphoric Acid used were of Analytical reagent grade. HPLC grade Acetonitrile and water was used throughout the experiment. Lacosamide oral solution, Analyte standard and all impurities standards were obtained from Hetero Labs Ltd (Hyderabad, India).

2.2 Equipment:

High performance Liquid chromatography system (from Waters) with auto sampler and with Photo Diode Array detector was used for the study. Data was acquired and processed by using Waters Empower software.

2.3 Chromatographic Conditions:

The analysis was carried out on Waters Symmetry C8, 250 x 4.6mm, 5µ column. The column oven temperature was maintained at 30°C. The mobile phase A consists of buffer, where buffer is prepared by dissolving about 1.36 g of

Potassium dihydrogen phosphate and 1.42 g of Di sodium hydrogen phosphate anhydrous to 1000 mL of water, pH adjusted to 2.0±0.05 with orthophosphoric acid. A mixture of water and acetonitrile in the ratio of 20:80 was used as Mobile phase B. The flow rate was set to 1.2 mL/minute in gradient elution mode. Gradient time program was set as T/%B: 0/5, 10/15, 20/20, 45/30, 55/60, 60/100, 70/100, 72/5, and 85/5.The injection volume was 10µL and the detection was performed at 210nm using a photo diode array (PDA) detector. The typical retention time of Lacosamide is about 25 minutes in the final optimized conditions. The criticality of this method is to elute impurities of Lacosamide with optimum separation and symmetric peak shapes with no interference due to placebo.

2.4 Sample Preparation:

2.4.1 Diluent Preparation:

Mixture of buffer and acetonitrile in the ratio of 90:10 %v/v.

2.4.2 Standard Preparation:

Accurately weighed and transferred about 25mg of Lacosamide working standard into a 200 mL volumetric flask added 120 mL of diluent and sonicated to dissolve, and volume was made up with diluent. Transferred 2.0 mL of above solution into a 50mL volumetric flask, diluted to volume with diluent and mixed well. A sensitivity standard solution of Lacosamide was also prepared by diluting 2.0 mL of standard solution to 20 mL volumetric flask, diluted to volume with diluent and mixed.

2.4.3 Preparation of Related compound-04 stock solution:

Accurately weighed and transferred about 2.0 mg of related compound-04 in to a 100 mL of volumetric flask, added about 60 mL of Acetonitrile and sonicated to dissolve. Diluted to volume with acetonitrile and mixed well.

2.4.4 Preparation of Resolution solution:

Accurately weighed and transferred about 50 mg of Lacosamide working standard into a 50 mL volumetric flask, added 5 mL of Related compound-04 stock solution. Dissolved and diluted to volume with diluent and mixed well.

2.4.5 Test Preparation:

Accurately transferred Oral solution equivalent to about 50 mg of Lacosamide into a 50mL volumetric flask. Added about 30 mL of diluent and kept on rotatory shaker for 5 min, diluted to volume with diluent and mixed well. Filtered a portion of the solution through 0.45µm membrane filter and discarded first few mL of the filtrate.

2.5 EXPERIMENTAL DESIGN:

2.5.1 Method Validation:

The aim of method validation was to confirm that the present method was suitable for its intended purpose as described in ICH guidelines [12]. The described method has been extensively validated in terms of specificity, precision, linearity, accuracy, Limit of Quantification, Robustness and solution stability. Specificity of the method was evaluated by injecting individual impurities and by subjecting the drug into forced stress conditions. The precision of the method was expressed in term of coefficient of variation (RSD) for % of impurities. The accuracy was expressed in terms of percent recovery of the known amount of impurities added to the sample preparation. To perform the validation activity Benzyl amine, DAM Impurity, Related compound-02, Related compound-03, Related compound-04, and Related compound-05 were selected.

RESULTS AND DISCUSSION

3.1 System suitability:

System suitability tests are an integral part of a liquid chromatographic method. As integral part of chromatographic method, system suitability parameters like USP Tailing, Theoretical plates and Relative standard deviation (RSD) for replicate injections of standard were evaluated and found to be satisfactory as per common chromatographic practices. Since it is related substances method it is necessary to monitor the sensitivity of the method and it was done by injecting a sensitivity standard of Lacosamide and by determining S/N ratio. According to the results presented, the proposed method fulfills these requirements within the accepted limits. Results are shown in Table No 1.

Table 1: Results of System Suitability Test

Name of Drug substances	Theoretical plates	USP Tailing factor	%RSD for replicate injections	S/N ratio
Lacosamide	62383	1	0.53	22

3.2 Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradents, matrix (placebo) etc. Specificity was tested by injecting the impurity standards individually, Test spiked with all impurities (Fig 2), placebo preparation and Forced degradation samples. Results of impurity interference study has been tabulated in Table No 2

Forced degradation studies were performed to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions like acid hydrolysis (using 1 N HCl), base hydrolysis (using 1 N NaOH), and oxidative degradation (using 3% H2O2) to evaluate the ability of the proposed method to separate degradation products from active ingredient. To check and ensure the homogeneity (peak purity) of peak in the stressed sample solutions, photo diode array detector was employed. In forced degradation it was observed that Lacosamide is susceptible to degradation in acid and base stress conditions. Peak purity in all the degradation conditions has been proven for Lacosamide peak. Results are tabulated in Table No 3.

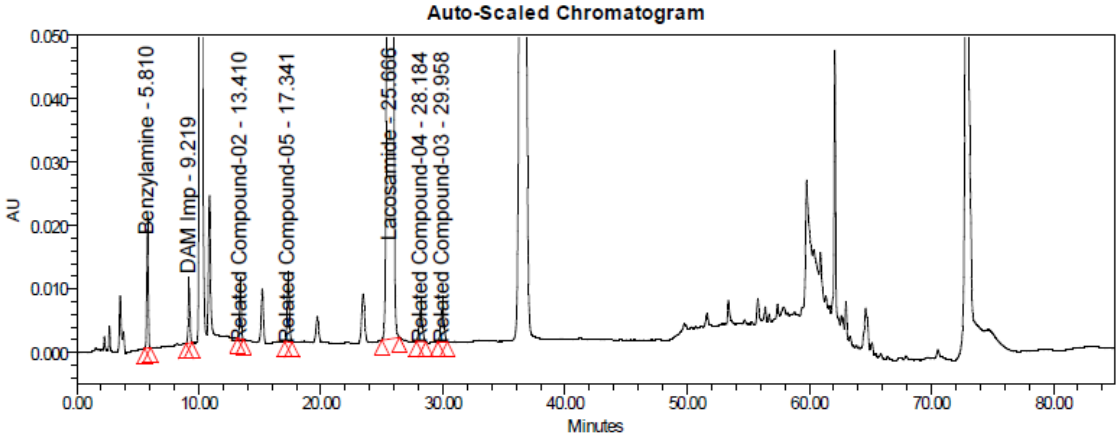


Fig. 2 Typical Chromatogram of Test Spiked with impurities

Table 2: Results of Specificity - Impurity Interference

Impurity Name	RT (minutes)
Benzyl amine	5.81
DAM Impurity	9.22
Related compound-02	13.41
Related compound-03	29.96
Related compound-04	28.18
Related compound-05	17.34
Lacosamide	25.67

Table 3: Results of Forced degradation Studies with Peak purity details

Stress Conditions	PA	PT	% Degradation
Acid Degradation	0.042	0.276	12.77
Base Degradation	0.088	0.276	17.77
Peroxide Degradation	0.049	0.284	0.65

PA = Purity Angle, PT= Purity Threshold

Note: Purity Angle should be less than Purity Threshold to meet Peak purity criteria acceptance criteria

3.3 Linearity:

The linearity of the method was tested in order to demonstrate proportional relationship of response versus impurities concentration over the working range. It is usual practice to perform linearity experiments over a wide range of impurity concentration covering from about LOQ to higher level. This gives confidence that the response and concentration are proportional and consequently ensures that calculations can be performed in the specified range. The linearity of detector response to different concentrations of all impurities of Lacosamide was studied by preparing a series of solutions. The data were subjected to statistical analysis using a linear-regression model. The results have indicated good linearity. Results are shown in Table No 4.

Table 4: Results of Linearity Studies (Response Vs Concentration)

Name of Impurities	Correlation coefficient	Intercept	Slope
Benzyl amine	0.999	570.1	34527.8
DAM Impurity	0.999	298.5	16662.4
Related compound-02	0.999	409.2	16450.8
Related compound-03	0.999	595.2	19776.1
Related compound-04	0.999	1431.6	16812.4
Related compound-05	0.999	1113.6	25880.3

3.4 Precision:

Six sample solutions (single sample Lot of Lacosamide oral solution) were prepared spiking the known impurities and the precision of the method was tested. The % RSD was calculated for results of all impurities and it indicates that proposed method has got acceptable level of repeatability. Results are tabulated in Table No 5.

Table 5. Method Precision data

Impurity Names	Mean from Six samples (% Impurity)	% RSD for six samples
Benzyl amine	0.500	0.72
DAM Impurity	0.472	0.70
Related compound-02	0.509	1.81
Related compound-03	0.519	0.81
Related compound-04	0.541	0.67
Related compound-05	0.489	0.65

3.5 Accuracy:

Accuracy of the proposed method was established by recovery experiments. This study was employed by spiking of known amounts of impurities into samples of at 0.05%, 100% and 150% of targeted concentration, in triplicate and injected into the chromatographic system. The resulting mixtures were analyzed as described in proposed method. Results obtained from recovery studies are given in Table No 6.

Table 6: Results of Recovery Study at Different Levels

Name of Impurity	0.05% level	100% level	150% level
Benzyl amine	112.1	95.1	95.4
DAM Impurity	95.0	89.5	88.7
Related compound-02	101.1	94.3	95.6
Related compound-03	105.0	96.6	97.0
Related compound-04	92.9	89.8	89.7
Related compound-05	97.1	96.3	96.5

Note: Number of samples analyzed at each level is in triplicate

3.6 Limit of Quantification (LOQ):

The limit of quantification for Lacosamide was determined by signal to noise ratio method. 0.05% was considered as theoretical LOQ (based on reporting threshold) and it was found that at 0.05% level signal to noise ratio was more than 10. Hence 0.05% was considered as LOQ.

3.7 Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters, and provides an indication of its reliability during normal usage. In the present study, an experimental design was planned for robustness testing varying some conditions, e.g. Flow rate, column temperature, variation of buffer pH and change in mobile phase B composition. As this is related substances method critical parameters like Resolution and RRT's of impurities were measured. It can be seen that, with every employed condition, there were no dramatic changes in the chromatographic behavior. The results are shown in Table No 7.

Table 7: Results of Robustness Study

Parameter	Deliberate change	Resolution	RRT					
			Benzyl amine	DAM Impurity	RC-2	RC-5	RC-4	RC-3
Flow rate (1.2mL/min)	1.0mL/min	3.3	0.21	0.37	0.60	0.74	1.08	1.16
	1.5mL/min	2.9	0.19	0.22	0.57	0.73	1.07	1.14
Temperature (30°C)	35°C	2.8	0.20	0.26	0.56	0.74	1.07	1.15
pH of buffer (2.0)	1.8	3.2	0.21	0.33	0.58	0.74	1.07	1.14
	2.2	3.5	0.22	0.29	0.56	0.73	1.08	1.15
MP B (20:80)	20:72	2.9	0.23	0.32	0.55	0.73	1.07	1.14
	20:88	3.2	0.25	0.30	0.56	0.72	1.07	1.15

3.8 Solution Stability:

Solution Stability studies were performed for Standard and test spiked solution for about 24 hours at bench top. Results from this study were compared against initial standard area and % impurity for test solution. Data indicates that both standard and test solutions are bench top for about 24 hours with acceptance criteria of ± 0.05 for impurities and ±5 % for standards. Results obtained from solution stability studies are given in Table No 8.

Table 8: Results of Solution Stability Studies

Name	Initial	After 24 Hours	Difference from the Initial
Standard for Lacosamide	117313	113076	3.6%
Benzyl amine	0.492	0.495	0.003
DAM Impurity	0.466	0.469	0.003
Related compound-02	0.494	0.530	0.036
Related compound-03	0.511	0.505	0.006
Related compound-04	0.535	0.530	0.005
Related compound-05	0.480	0.487	0.007
Total impurities	2.978	3.016	0.038

CONCLUSION

The novel gradient RP-HPLC method developed for quantitative analysis of impurities of Lacosamide in oral solution pharmaceutical dosage form is accurate, precise, linear, robust and specific. Satisfactory results were obtained during method validation experiments. This method is suitable for the routine analysis of production samples to check the quality of the product with respect to impurities.

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