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Stability indicating RP-UPLC method for the determination of Lacosamide and its impurities in Bulk drugs and its pharmaceutical dosage forms

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ABSTRACT

A novel stability-indicating reverse phase Ultra performance liquid chromatography method was developed and validated for the determination of lacosamide (LCM) and its related substances in bulk and pharmaceutical formulations. The separation of impurities from lacosamide was accomplished on HSS C18 Column using (100 x 2.1mm, 1.8µm) 0.01 M mono basic potassium phosphates for adjusting the pH to 2.0 with ortho phosphoric acid: Acetonitrile (85:15, v/v) as mobile phase. The flow rate was 0.7 mL/min and the detection was carried out at 210 nm with run time of 5 minutes. The developed isocratic UPLC method was consequently validated for specificity, linearity, range, accuracy, precision and robustness and shown equivalency with the API Vendor method.

Key words: Lacosamide, RP-HPLC, Degradation products, Stability indicating.

INTRODUCTION

Lacosamide newly licensed drug was used in the treatment of diabetic neuropathic pain and partial onset seizures in adults with epilepsy. It is a functionalized amino acid with a novel mechanism of action. It possesses excellent oral absorption, negligible protein binding, minimum interaction with other antiepileptic drugs and is excreted mainly in the urine. Epilepsy is a major neurological disorder, affecting up to 2% of the population worldwide and each year more than 100,000 new cases are diagnosed in US [1-7] and also number of cases found in India. Lacosamide drug was approved by United States Food and Drug Administration (FDA) in the year 2007.

The drug shows electrophysiological characters, modulates some voltage-gated sodium channels interacting with slow inactivated sodium channels and binding with collapsing response mediator protein (2) [8]. The chemical name of lacosamide is (2R)-2-(acetyl amino)-N-benzyl-3-methoxypropanamide (C13H18N2O3).

The literature survey reveals that there are available HPLC Methods [9-16], UV Spectroscopic methods [15-17], Furthermore, to the best of our knowledge; no stability-indicating UPLC method is reported in the literature.

The objectives of the present manuscript describe the degradation behaviour of lacosamide under hydrolysis (acid, base and neutral), oxidation, thermal and photolysis conditions. To optimize the liquid chromatography conditions to separate the drug from its degradation products on a reverse phase HSS, C18 column and to establish a validated stability-indicating Assay and its impurities method by UV detection at 210 nm. The developed UPLC method was validated as per the International Conference on Harmonization (ICH) guidelines [18-19].

MATERIALS AND METHODS

2.1 **Instrumentation:**
A Waters Acquity Ultra Performance liquid chromatography equipped with PDA Detector with Binary pump. The column utilised was Acquity UPLC HSS C-18, 2.1x100mm, 1.8µm.

2.2 **Materials:**
Lacosamide working standard and all four related substances (Table1) were produced from MSN Laboratories, Hyderabad. Acetonitrile and Potassium di hydrogen phosphate from Merck. , Vimpat tablets were obtained from UCB Pharma, SA.

The compounds related to Lacosamide which could be expected as impurities or might appear as degradation products have been prepared and identified by MSN Laboratories and listed in below table.

Table: 1 Impurities and their chemical names.

Name	Chemical Name
Amino impurity (Impurity-01)	(R)-2-Amino-N-benzyl-3-methoxypropionamide, Molecular formulae:$C_{11}H_{16}N_2O_2$, Molecular weight: 208.26. This is a process related impurity
Hydroxy impurity (Impurity-02)	(R)-2-Acetamido-N-benzyl-3-hydroxypropanamide. Molecular formulae: $C_{12}H_{16}N_2O_3$, Molecular weight: 236.27. This is a process related impurity
Acetamide impurity (Impurity-03)	N-Benzyl acetamide. Molecular formulae: $C_9H_{11}NO$, Molecular weight: 149.19. This is a process related impurity
O-Acetyl impurity (Impurity-04)	(R)-2-Acetamido-3-(benzyl amino)-3-oxopropyl acetate. Molecular formulae: $C_{14}H_{18}N_2O_4$, Molecular weight: 278.3. This is a process related impurity

2.1 **Developing an UPLC Method:**
The UPLC method carried out in this study aimed at developing a chromatographic system capable of eluting and resolving Lacosamide and its impurities (related substances) from one another and that complies with the general requirements for system suitability.

All the Development related trails and observations are summarized and in below table.

Table: 2 Method development trails and observations.

Trail No	Changes in trail	Results
1	Column: BEH C-18, 2.1x100mm, Acetonitrile and 0.01 M Sodium di hydrogen phosphate(70:30)	The resolution Impurity -3 and Lacosamide was low.
2	Used methanol as organic modifier instead of Acetonitrile.	High tailing factor and longer retention times.
3	Changed the column to HSS,C-18,100mm	Tailing factor was reduced.
4	Changed the buffer to 85%	Enhancement of peak symmetry and Increase in resolution with impurity-03
5	When the pH was 3 to 7	Decrease in retention times and resolution was poor.

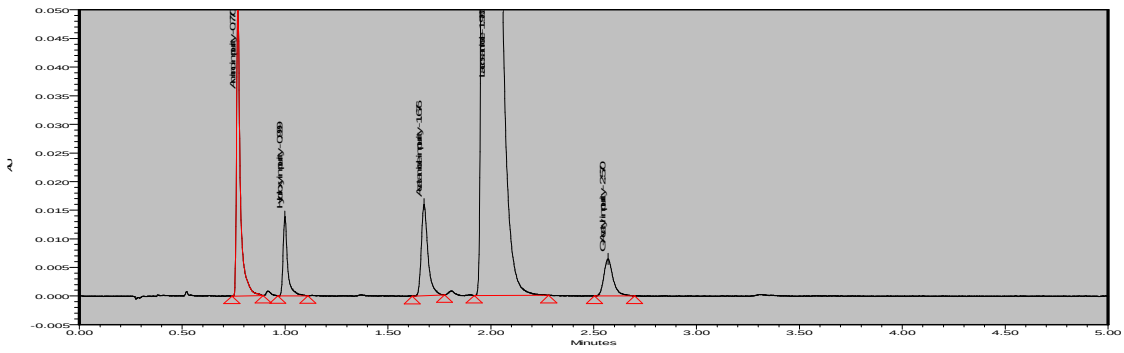

Fig: 1: Impurity spiked sample:

2.2 Finalized conditions:
Isocratic elution technique was utilized with the column maintained at 45°C the Buffer phase used was Potassium di hydrogen ortho phosphate [0.01M] adjusted to pH 2.0 with dilute ortho phosphoric acid as aqueous phase and

Acetonitrile was used as organic phase in 85: 15 ratio. The flow rate was 0.7 mL/min. Samples of 1µL was injected into the column and the detector was set at 210 nm with run time of 5 minutes. The relative standard deviation (R.S.D.) of six replicate injections of the standard preparation was not greater than 2.0% and the tailing factor was less than 2.0.

2.3: Preparation of solutions:

2.3.1 Preparation of diluted standard solution

An accurately weighed quantity of Lacosamide or related substances (Impurities 1, 2, 3 and 4) was dissolved in the diluent (water: Acetonitrile in 80:20 ratio) and diluted quantitatively. Serial dilutions were carried out, using the diluent, to obtain solutions of known concentrations to be used for the standard reparation (5 ppm for Impurities and 200 ppm for assay).

2.3.2: Preparation of Test solutions (Vimpat):

Twenty tablets were weighed and powdered and accurately weighed portions equivalent to 50 mg Lacosamide were transferred to 50 ml volumetric flasks. Disintegrated with 30 mL of Diluent was added up to the volume. The solutions were sonicated and centrifuged as above and the supernatant was used as test solution for impurities with 1.0 mg per ml further transfer 5 mL of the above solution to 25 mL with the diluent for Assay (200 ppm).

2.4 Quantification:

Equal volumes, (1µL), of the standard preparations and the test preparations that contain Lacosamide in the diluent were injected into the chromatograph and the quantified for Lacosamide and its impurities.

2.5 Linearity, Limit of detection and limit of quantification:

Calibration graphs were constructed for Lacosamide and its impurities in either standard solution .The degree of linearity was assessed by the correlation coefficient, y-intercept, and slope. The limit of detection, LOD and the limit of quantitation LOQ have been estimated as 3 S.D. and 10 S.D. of the y intercept and slope.

2.6 Precision:

The precision was performed by preparing six individual preparations as per the method of analysis and evaluated for percentage of Lacosamide and its individual impurities and percentage of total impurities.

2.7 Accuracy:

The samples were prepared by spiking the Active substances and impurities stock solutions into the drug placebo mixture and the percent recovery was estimated.

2.8: Solution stability:

The solutions prepared was tested at initial, 24hrs and 48Hrs by maintaining at room temperature and estimated for Lacosamide impurity content.

2.9: Robustness:

Robustness was conducted by making the variations in flow rate, Column oven temperature and percentage of Acetonitrile.

2.10: Ruggedness:

The prepared solutions were filtered through 0.45 µ PVDF syringe filter and 0.45 µ PTFE syringe filter and evaluated difference between the lacosamide and impurities content.

2.11: Intermediate precision:

The test was performed with another analyst on different day, different system and different column and the lacosamide and its impurity contents were reported.

2.12: Forced degradation studies:

The forced degradation studies conditions and % degradation s mentioned in the results (Table: 8) section.

RESULTS AND DISCUSSION

Table: 3: Validation characteristics of Assay method

Parameter	Results
Specificity	Blank interference, Placebo interference, Impurity interference was nil.
Linearity	Established from 20 ppm to 600 ppm (R^2 value=0.9998)
Precision:	% RSD of impurity for six preparations= 0.49
Accuracy	**%Level** **%Recovery** 20% 99.4 50% 99.1 80% 100.1 100% 100.5 120% 99.8
Solution stability	1)% Difference at 24 Hrs=0.12% 2)%Difference at 48 Hrs=0.23%
Robustness	Flow rate variation-System suitability passes Temperature variation system suitability passes
Ruggedness	Filter validation: Variation between PVDF &PTFE 0.45 micron filters=0.11%
Intermediate precision	%RSD=0.3%

Table: 4: Validation characteristics of Amino impurity.

Parameter	Results
Response factor	0.89
Specificity	Blank interference, Placebo interference, Impurity interference was nil.
Linearity	Established from 0.4 ppm to 21 ppm (R^2 value=0.9995)
LOD and LOQ	LOD=1 ppm and LOQ=2.1 ppm
Precision:	% RSD of impurity for six preparations= 3.02
Accuracy	**%Level** **%Recovery** 10% 88.8 20% 97.3 30% 89.8 50% 98.8 100% 101.0 400% 110.2
Solution stability	1)% Difference at 24 Hrs=0.01% 2)%Difference at 48 Hrs=0.03%
Robustness	Flow rate variation-System suitability passes Temperature variation system suitability passes
Ruggedness	Filter validation: Variation between PVDF &PTFE 0.45 micron filters=0.01%
Intermediate precision	Individual impurity variation=0.03% Total impurity variation=0.03%

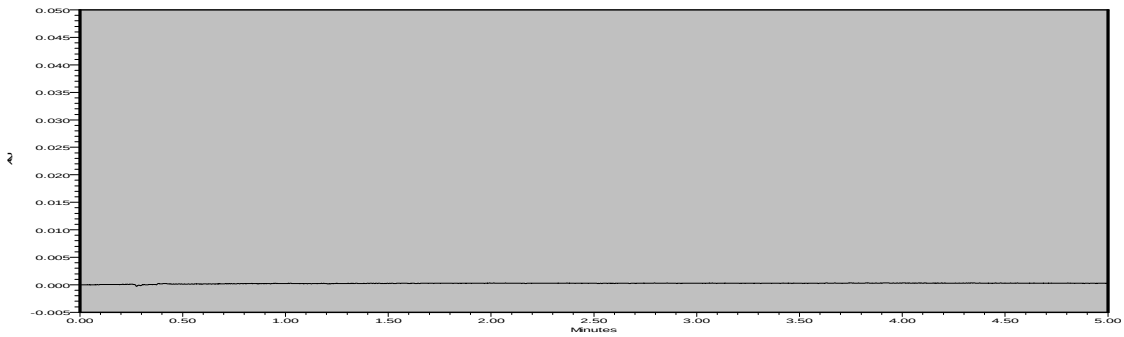

Fig: 2: Blank chromatogram

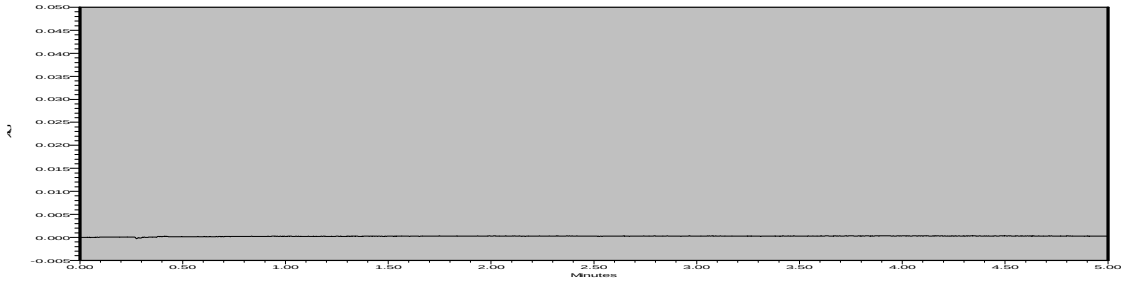

Fig: 3: Placebo chromatogram

Table: 5: Validation characteristics of Hydroxy impurity

Response factor	0.98
Specificity	Blank interference, Placebo interference, Impurity interference was nil.
Linearity	Established from 0.4 ppm to 21 ppm (R^2 value=0.9995)
LOD and LOQ	LOD=1.29 ppm and LOQ=3.91 ppm
Precision:	% RSD of impurity for six preparations= 0.81
Accuracy	**%Level** **%Recovery** 10% 93.1 20% 101.6 30% 107.8 50% 100.1 100% 95.8 400% 91.6
Solution stability	1)% Difference at 24 Hrs=0.01% 2)%Difference at 48 Hrs=0.03%
Robustness	Flow rate variation-System suitability passes Temperature variation system suitability passes
Ruggedness	Filter validation: Variation between PVDF &PTFE 0.45 micron filters=0.01%
Intermediate precision	Individual impurity variation=0.01% Total impurity variation=0.03%

Table: 6: Validation characteristics of Acetamide impurity

Response factor	1.32
Specificity	Blank interference, Placebo interference, Impurity interference was nil.
Linearity	Established from 0.4 ppm to 21 ppm (R^2 value=1)
LOD and LOQ	LOD=0.03 ppm and LOQ=0.08 ppm
Precision:	% RSD of impurity for six preparations= 0.22
Accuracy	**%Level** **%Recovery** 10% 99.4 20% 100.5 30% 100.5 50% 100.2 100% 98.2 400% 99.0
Solution stability	1)% Difference at 24 Hrs=0.00% 2)%Difference at 48 Hrs=0.01%
Robustness	Flow rate variation-System suitability passes Temperature variation system suitability passes
Ruggedness	Filter validation: Variation between PVDF &PTFE 0.45 micron filters=0.01%
Intermediate precision	Individual impurity variation=0.02% Total impurity variation=0.03%

Table: 7: Validation characteristics of O-Acetyl impurity

Response factor	0.91
Specificity	Blank interference, Placebo interference, Impurity interference was nil.
Linearity	Established from 0.4 ppm to 21 ppm (R^2 value=1)
LOD and LOQ	LOD=0.2 ppm and LOQ=0.61 ppm
Precision:	% RSD of impurity for six preparations= 2.41
Accuracy	**%Level** **%Recovery** 10% 110.3 20% 108.0 30% 108.3 50% 98.6 100% 95.2 400% 90.6
Solution stability	1)% Difference at 24 Hrs=0.02% 2)%Difference at 48 Hrs=0.02%
Robustness	Flow rate variation-System suitability passes Temperature variation system suitability passes
Ruggedness	Filter validation: Variation between PVDF &PTFE 0.45 micron filters=0.01%
Intermediate precision	Individual impurity variation=0.02% Total impurity variation=0.03%

Table: 8: Forced degradation Results

Type	Condition &Duration	% Degradation	Peak purity	%Assay
Acid	1N Hcl,24Hrs,50°C	8.61	Passes	91.2
Base	1N NaoH,24Hrs,50°C	7.46	Passes	92.6
Peroxide	10% H_2O_2,24Hrs,50°C	1.32	Passes	98.3
Water	Water,24Hrs,50°C	0.12	Passes	99.7
Thermal	24Hrs,50°C	0.09	Passes	99.0
Photo	1.2 million Lux hours	0.09	Passes	99.1

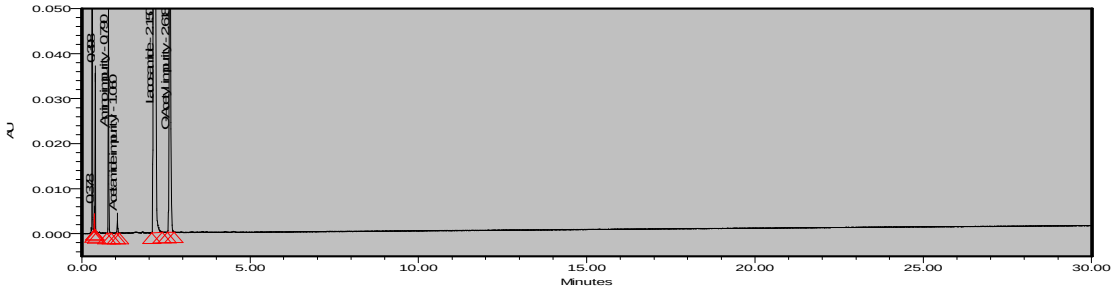

Fig: 4: Acid stressed sample chromatogram

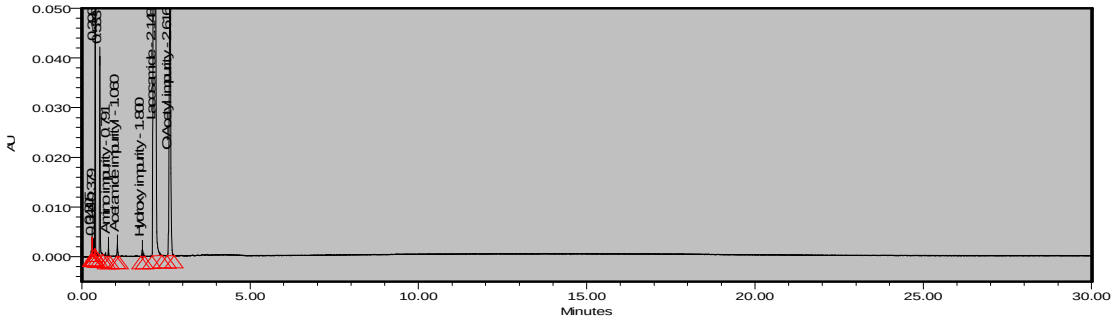

Fig: 5: Base stressed sample chromatogram

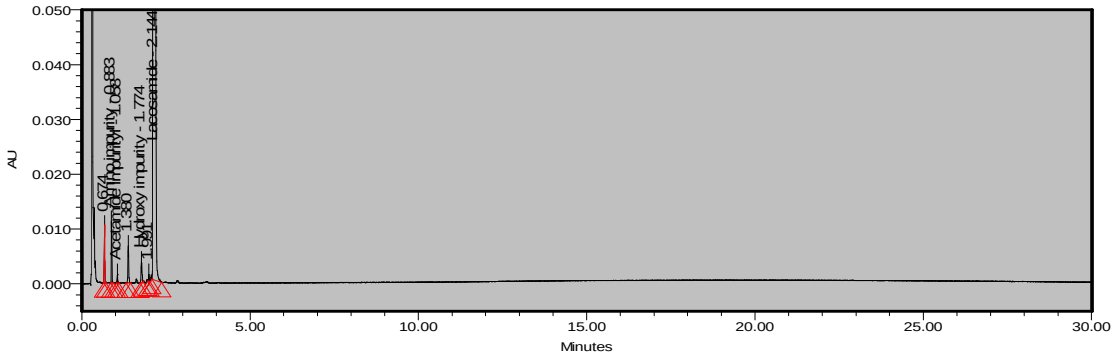

Fig: 6: Peroxide stressed sample chromatogram

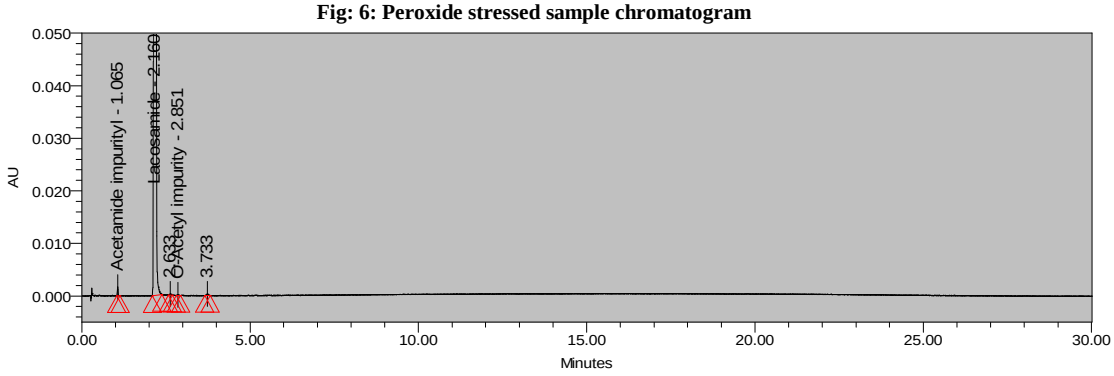

Fig: 7: Water stressed sample chromatogram

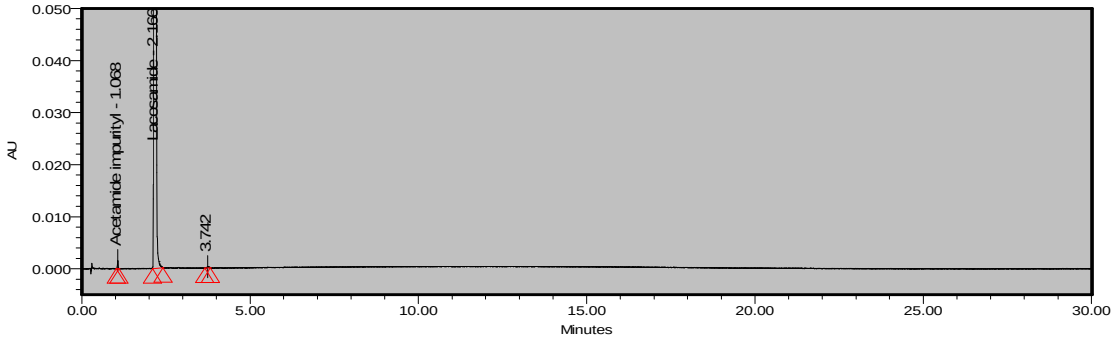

Fig: 8: thermal stressed sample chromatogram

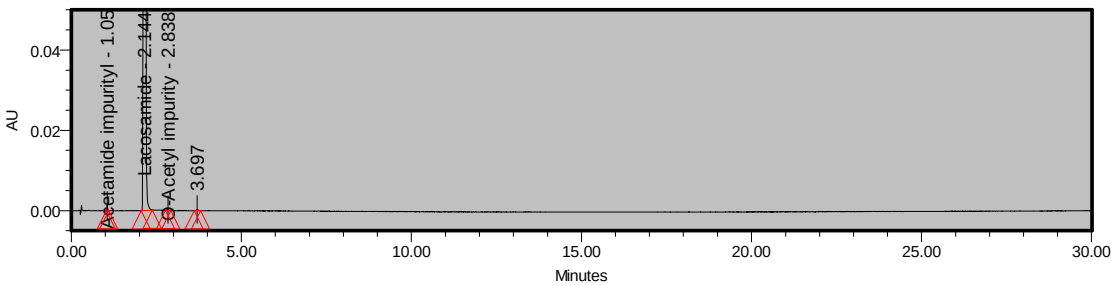

Fig: 9: Light stressed sample chromatogram

3.8 Application to Vimpat:

The validity of the method developed here for quantification of Lacosamide and the impurities that might interfere in the determination of Lacosamide was studied by assaying a commercial Lacosamide product and Vimpat tablets (Manufacturer UCB Pharma, SA). Two of the compounds related to Lacosamide appeared clearly on the chromatogram; this indicates that the proposed method can differentiate between the active moiety and its related impurities. Samples of Vimpat® were analysed for Lacosamide and its impurities by this method and the results showed.

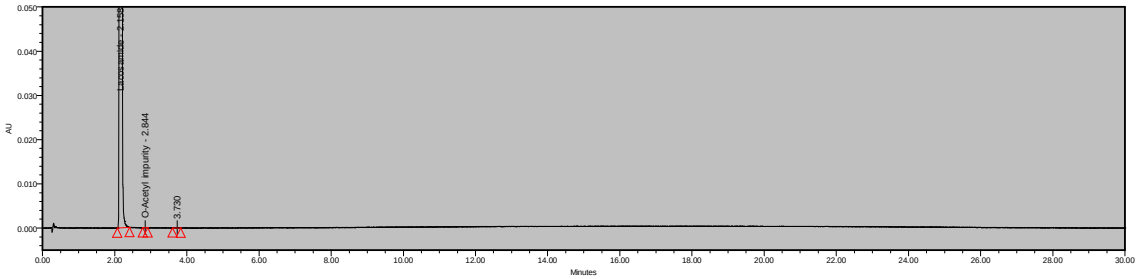

Fig: 10: Vimpat sample chromatogram

3.9 Study for un-eluted peaks:

Since the runtimes are lower, a study conducted on all the stressed samples for knowing the retained peaks by increasing the Acetonitrile to 90% for 30 minutes. The results showed there was no peak eluted at all.

3.10 Equivalency study with HPLC method:

The developed UPLC Method was compared with HPLC method (Obtained from API Vendor) results.

Table 9: System suitability equivalence.

S.No	Method	Parameter	Criteria	HPLC method Result	Proposed Method
1	Assay	Tailing factor	Not more than 2.0	1.34	1.04
2	Assay	Standard %RSD	Not more than 1.0	0.31	0.25
3	Impurities	Tailing factor for LCM- Peak	Not more than 2.0	1.54	1.17
4	Impurities	Resolution between Lacosamide and Acetamide impurity	Not Less than 2.0	2.4	3.1

Table10: API Batch analysis Results (B.NO:LS0010411)

S.No	Method	Criteria	HPLC method Result	Proposed Method
1	Assay	98.0-102.0%	99.8	99.9
2	Impurities			
3	Maximum individual impurity	Not more than 0.15%	0.03	0.03
4	Total impurity	Not more than 0.3%	0.05	0.05

Table11: VIMPAT Tablet analysis Results (B.NO:8444804)

Details	HPLC method Results	UPLC Method results
Known impurity	1)0.01%	1)0.01%
Any unknown individual impurity	2)0.05%	2)0.05%
Total impurity	3)0.08%	3)0.09%

CONCLUSION

An UPLC method for related compounds in the commercial drug products and in the tablet formulation was validated in this study. Lacosamide and its impurities which may co exist with it as impurities or as degradants gave chromatograms of very well resolved peaks which indicate the specificity of the method and the possibility of using it as an indicator of stability. Slight changes in the experimental conditions did not affect significantly the resolution of the compounds of interest or their percent recoveries indicating the robustness of the method. All the statistical values (percent recovery, RSD, %D, the slope and the intercept, LOD and LOQ) calculated were within the acceptable limits. The method was shown to be equivalent with the HPLC Vendor method with the run time of 10 minutes. It can be used for estimation of Lacosamide and its related substances in bulk drugs, solid dosage form and quality control purposes.

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