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# DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF LACOSAMIDE AND ITS RELATED SUBSTANCE IN PARENTERAL DOSAGE FORM

**Ankita Y. Chauhan*, Dr. C. J. Patel[1] and Dr. M. M. Patel[2]**

[*]Department of Pharmaceutical Quality Assurance, Shree Swaminarayan Sanskar Pharmacy College, Gandhinagar, Gujarat, India.

[1]Professor, Department of Pharmaceutical Quality Assurance, Shree Swaminarayan Sanskar Pharmacy College, Gandhinagar, Gujarat, India.

[2]Principal, Shree Swaminarayan Sanskar Pharmacy College, Gandhinagar, Gujarat, India.

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***Corresponding Author**
**Ankita Y. Chauhan**
Department of Pharmaceutical Quality Assurance, Shree Swaminarayan Sanskar Pharmacy College, Gandhinagar, Gujarat, India.

## ABSTRACT

A reverse phase high performance liquid chromatography method has been developed and subsequently validated for determination of lacosamide and its related substance in parenteral dosage form. Chromatographic separation was achieved in gradient mode with Ascentis express $C_{18}$ (50 mm x 4.6mm I.D, particle size 2.7µm) column with mobile phase A containing buffer and methanol in the ratio 92:8 v/v. The buffer was prepared by diluting 1 ml of ortho-phosphoric acid in 1000 ml of purified water. Mobile phase B containing Acetronitrile as eluent at a flow rate of 1.0 ml/min and total elution time was 50 minutes. The UV detection was performed at 215 nm and column temperature was 30ºC. The injection volume is 10µl. Lacosamide was subjected to acid and alkali hydrolysis, and peroxide degradation. Also the degraded products were well separated from the pure drug. The linearity observed for amino impurity is in the range of 0.20-15.17 µg/ml and for desmethoxy impurity is 0.20-3.11 µg/ml. The recovery was found to be in the range of 100.6-101.3 % for amino impurity and 90.2-93.96 % for desmethoxy impurity. RSD % Recovery should not be more than 5. The proposed method was successfully applied for the determination of lacosamide and its related substance in routine quality control analysis in parenteral dosage form.

**KEYWORDS:** Lacosamide, method development, RP-HPLC, Validation.

## INTRODUCTION

Lacosamide is a functionalized amino acid that has activity in the maximal electroshock seizure test, and is indicated for the adjunctive treatment of partial onset seizures and diabetic neuropathic pain.[1] The chemical name of lacosamide is ®-2-acetamido-N-benzyl-3-methoxypropionamide. Its molecular formula is $C_{13}H_{18}N_2O_3$ and its molecular weight is 250.30g/mol.[2] Lacosamide is white to light yellow powder. It is sparingly soluble in water and slightly soluble in acetonitrile and ethanol. The drug shows electrophysiological characters that modulates some voltage gated sodium channels and binding with collapsing response mediator protein. There are several process or degraded impurities associated with synthesis of lacosamide. Five of the known lacosamide related substance have mentioned here namely, amino alcohol impurity, amino impurity, acetamide impurity, o-acetyl impurity and desmethoxy impurity.[3] It is not official in any pharmacopoeia, few chromatographic methods have been reported for the determination of impurities in lacosamide.[4-10] In the proposed method related impurities are well separated and eluted before 50 minutes. Hence the developed method was consequently validated in terms of linearity, accuracy, precision and robustness for the determination of lacosamide and its related substance in parenteral dosage form.[11,12]

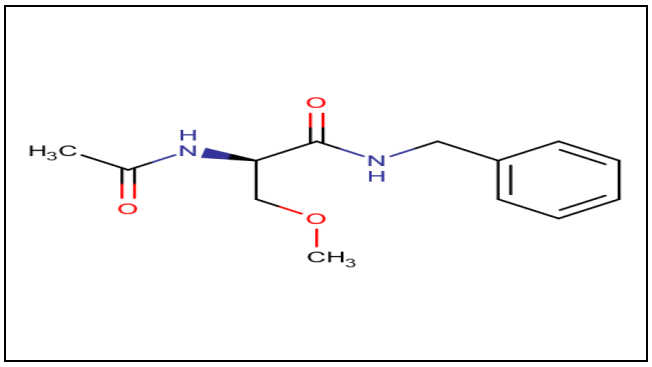


**Fig. 1: Structure of lacosamide.**

## MATERIAL AND METHODS

### Chemical and Reagents

Working standard and sample (Lacosamide Injection 10mg/ml) was provided by Zydus Cadila, Moraiya, Gujarat, India. Acetonitrile and Ortho-phosphoric acid are of HPLC grade manufactured by Merck life sciences Pvt. Ltd. Mumbai and other reagents used in study were of analytical grade.

**Instrumentation**

The analysis of drug was carried out on HPLC Agilent-1200 series with PDA detector. The column used in the development is Ascentis express $C_{18}$ (50 mm x 4.6mm I.D, particle size 2.7µm). The output of signal was monitored and integrated using Chromeleon software.

**Chromatographic Condition and Parameters**

Buffer: Take 1 ml of ortho phosphoric acid in 1000 ml of purified water.

Mobile phase A: Buffer: MeOH (92:8) %v/v

Mobile phase B: ACN (100) %v/v

Column: Ascentis Express $C_{18}$ (50mm x 4.6mm, 2.7µm)

Flow rate: 1.00 ml/min

Wavelength: 215 nm

Injection Volume: 10 µl

**Table 1: Gradient Program.**

| Time (min) | MP A % v/v | MP B % v/v |
|---|---|---|
| 0 | 100 | 0 |
| 20 | 100 | 30 |
| 35 | 30 | 70 |
| 40 | 30 | 70 |
| 42 | 100 | 0 |
| 50 | 100 | 0 |

**Mobile phase- MP*

**Preparation of solutions**

**Preparation of Diluent**

Distilled water is used as a diluent.

**Preparation of standard solution (2 ppm)**

Accurately weigh and transfer about 50 mg of lacosamide working standard into 50 ml volumetric flask, add 30 ml of diluent and sonicate to dissolve and dilute to volume with diluent, further dilute 5.0 ml of this solution to 50.0 ml with diluent. Further dilute 2.0 ml of this solution to 100.0 ml with diluent.

**Placebo preparation**

Transfer an accurately 5.0 ml of the placebo in 50 ml of volumetric flask. Add about 30 ml of diluent and mix. Make volume upto mark with diluent and mix.

**Sample Preparation (1000ppm)**

Transfer an accurately 5.0 ml of the sample in 50 ml of volumetric flask. Add about 30 ml of diluent and mix. Make volume upto mark with diluent and mix.

**Individual impurity preparation**

**Impurity stock solution preparation (100 ppm)**

Take 1 mg of amino-alcohol impurity, acetamide impurity, O-acetyl impurity and desmethoxy impurity into 10 ml of volumetric flask individually, make up the volume with the diluent and mix well.

**Amino impurity stock solution preparation (500 ppm)**

Take 5 mg of amino impurity in 10 ml of volumetric flask and then make up with diluent and mix well.

**Amino impurity (10 ppm)**

Take 0.4 ml of amino impurity from amino stock solution in 20 ml of volumetric flask, make up the volume with the diluent and mix well.

**Amino alcohol impurity (2 ppm)**

Take 0.4 ml of impurity solution from stock solution in 20 ml of volumetric flask, make up the volume with the diluent and mix well.

**Acetamide impurity (2 ppm)**

Take 0.4 ml of impurity solution from stock solution in 20 ml of volumetric flask, make up the volume with the diluent and mix well.

**O-Acetyl impurity (2 ppm)**

Take 0.4 ml of impurity solution from stock solution in 20 ml of volumetric flask, make up the volume with the diluent and mix well.

**Desmethoxy impurity (2 ppm)**

Take 0.4 ml of impurity solution from stock solution in 20 ml of volumetric flask, make up the volume with the diluent and mix well.

**Spiked Impurities Mixture (Specification limit of Impurities: 1.0% and 0.2%)**

**(1000 ppm of sample +10 ppm of amino impurity +2 ppm of other impurities)**

Transfer an accurately 2 ml of lacosamide sample solution in 20 ml of volumetric flask and add 0.400 ml of amino impurity from amino stock solution and 0.400 ml of amino alcohol impurity, acetamide impurity, O-acetyl impurity and desmethoxy impurity from respective impurity stock solution. Make up with the diluent and mix well.

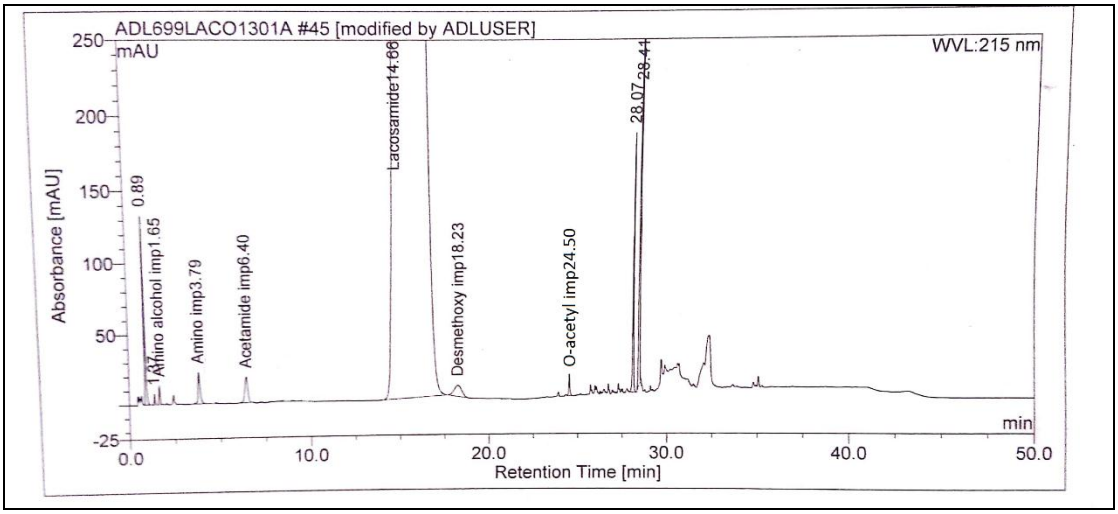


**Fig 2: Chromatogram of impurities mixture + API.**

**Table 2: System suitability parameters in optimized condition.**

| Retention time (min) | RRT* | Peak name | Plates | Tailing | Resolution |
|---|---|---|---|---|---|
| 1.65 | 0.11 | Amino alcohol impurity | 5116 | 1.14 | - |
| 3.79 | 0.26 | Amino impurity | 7450 | 0.96 | 9.22 |
| 6.4 | 0.43 | Acetamide impurity | 6482 | 1.02 | 7.01 |
| 14.66 | 1.0 | Lacosamide | 9226 | 0.96 | 11.69 |
| 18.23 | 1.24 | Desmethoxyimpurity | 7911 | 1.01 | 2.33 |
| 24.50 | 1.67 | O-Acetyl impurity | 6116 | 1.40 | 18.32 |

**Relative retention time-RRT*

## FORCED DEGRADATION

### Sample preparation for Acid Hydrolysis

Take accurately 1 ml of prepared sample stock solution into 10 ml of volumetric flask. Add 0.5 ml of 5 N HCl and shake. Keep the sample on water bath at 80ºC for 1 hour. Cool at room temperature. Then, the solution was neutralized with 5 N NaOH and and make up with diluent.

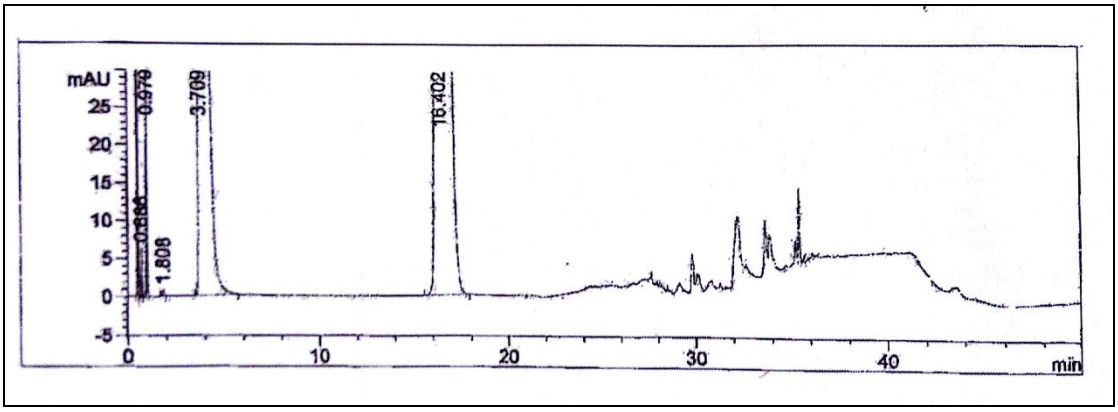


**Fig. 3: Sample Acid Degradation.**

**Sample preparation for Base Hydrolysis**

Take accurately 1 ml of prepared sample stock solution into 10 ml of volumetric flask. Add 0.5ml of 5 N NaOH and shake. Keep the sample on critical room temperature for 2 hours. Then, the solution was neutralized with 5 N HCl and make up with diluent.

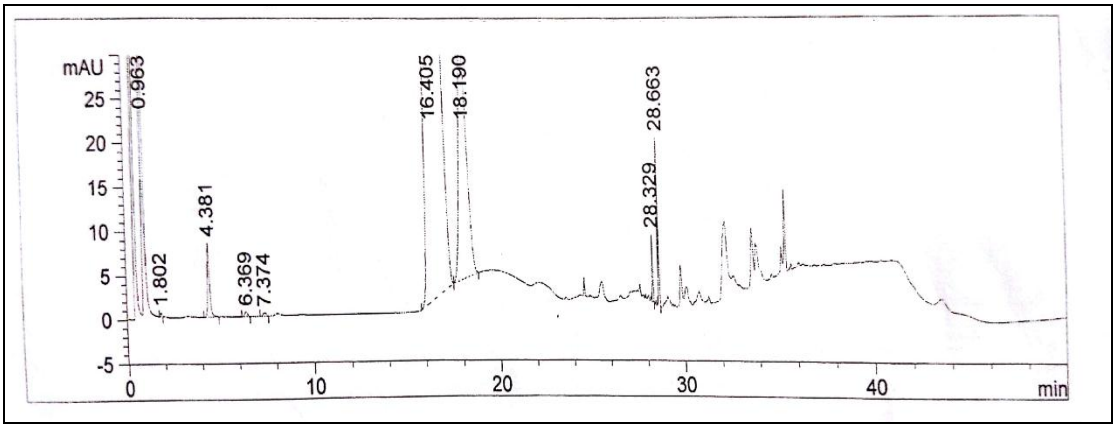


**Fig 4: Sample Alkali Degradation.**

**Sample preparation for Peroxide Oxidation**

Take accurately 1 ml of prepared sample stock solution into 10 ml of volumetric flask. Add 0.5 ml of 30% $H_2O_2$ and shake. Keep the sample on water bath at 80ºC for 1.5 hours. Cool at room temperature and make up with diluent.

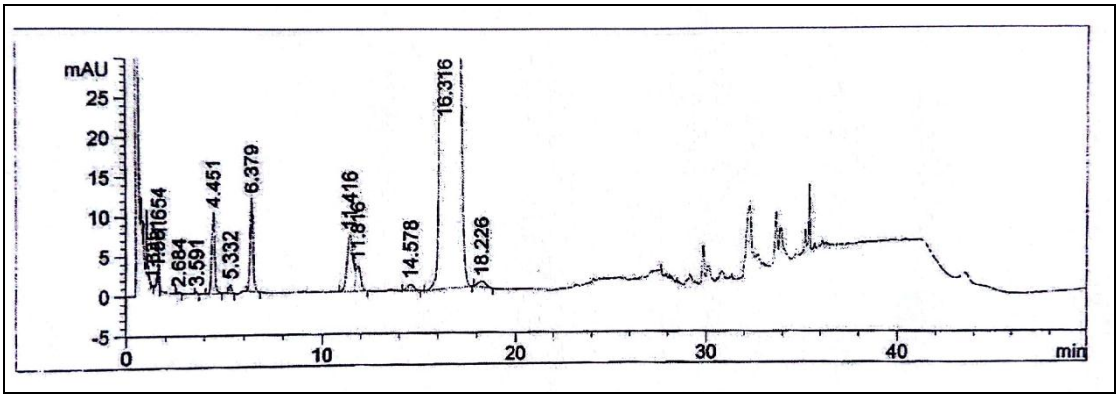


**Fig 5: Sample Peroxide Degradation.**

**Table 3: Degradation Summary.**

| Sr. No. | Stress Condition | Duration | Area | % Degradation | % Mass Balance |
|---|---|---|---|---|---|
| 1 | Acid Hydrolysis (5 N HCL_0.5ml) | 80ºC for 1 hour | 1661450 | 92.8 | 100.6 |
| 2 | Base Hydrolysis (5 N NaOH_0.5ml) | CRT for 2 hours | 1444975 | 80.8 | 111.5 |
| 3 | Peroxide Degradation (30% $H_2O_2$_0.5 ml) | 80ºC for 1.5 hour | 1750094 | 97.71 | 97.33 |

## METHOD VALIDATION

Specification limit of amino impurity and desmethoxy impurity (Not more than 1.0% and 0.2%).

## LINEARITY

According to specification limit, impurity should not be more than 1.0% and 0.2%, so solutions of linearity of impurity prepared as following in which 1.0% and 0.2% of sample concentration is considered as 100% that is 10 ppm and 2 ppm (sample concentration 1000 ppm). And from that consideration LOQ, 50%, 80%, 100%, 120% and 150% prepared.

**Table 4: Linearity result of Amino Impurity.**

| Level | Concentration (µg/ml) | Area |
|---|---|---|
| LOQ | 0.2017 | 2904 |
| 50 | 5.0578 | 67138 |
| 80 | 8.0925 | 110684 |
| 100 | 10.1156 | 139053 |
| 120 | 12.1387 | 165305 |
| 150 | 15.1734 | 207446 |

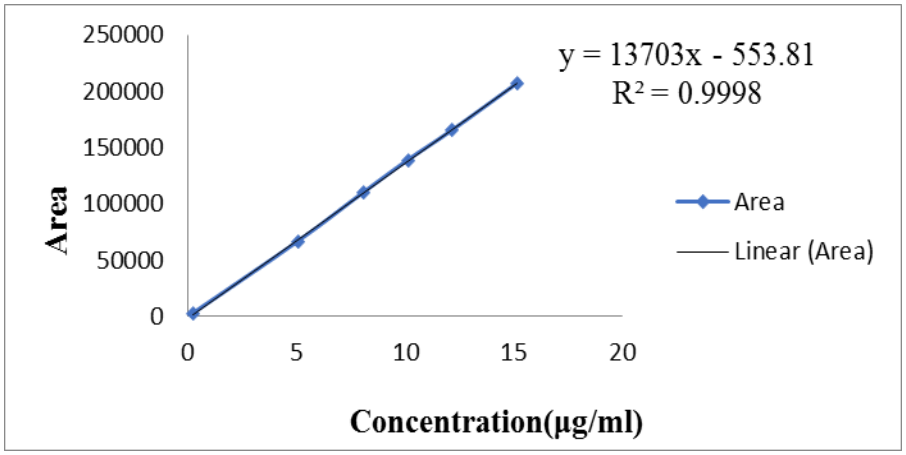


**Fig 6: Linearity curve of Amino Impurity.**

**Table 5: Linearity result of Desmethoxy Impurity.**

| Level | Concentration (µg/ml) | Area |
|---|---|---|
| LOQ | 0.2078 | 7815 |
| 50 | 1.039 | 38949 |
| 80 | 1.6624 | 62901 |
| 100 | 2.078 | 80538 |
| 120 | 2.4936 | 97308 |
| 150 | 3.117 | 122410 |

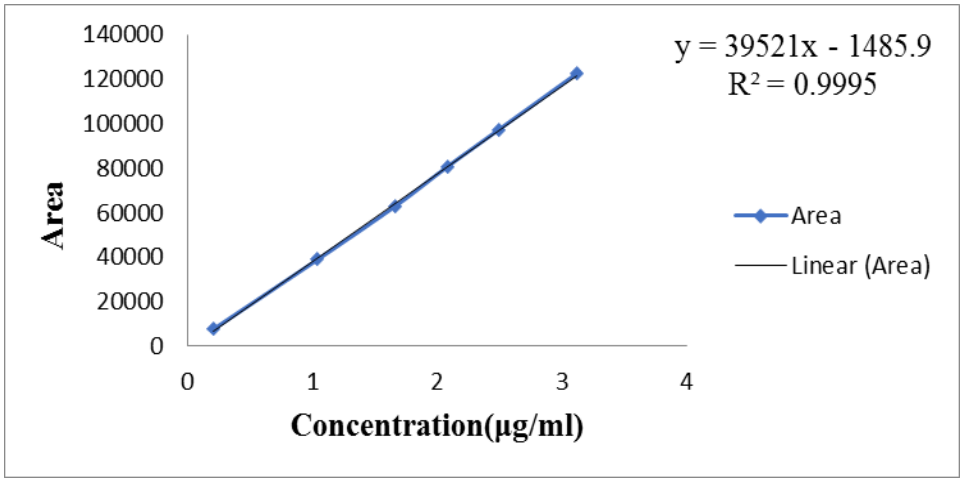


**Fig. 7: Linearity curve of Desmethoxy Impurity**

## ACCURACY

According to specification limit of impurities which is not more than 1.0% and 0.2%, amount of individual impurities will be 10 ppm and 2 ppm which is 1.0% and 0.2% of 1000 ppm (sample concentration). Accuracy is performed in sample at 4 levels which are LOQ, 50%, 100% and 150% in three sets.

**Table 6: Recovery result of Amino Impurity.**

| Set | Level | ml Added | mg Added | Area | mg found | % Recovery | % Mean Recovery | SD* | RSD* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | LOQ | 0.040 | 0.0042 | 7562 | 0.0039 | 92.9 | 93.96 | 1.15 | 1.23 |
| 2 | | 0.040 | 0.0042 | 7712 | 0.00394 | 93.8 | | | |
| 3 | | 0.040 | 0.0042 | 7763 | 0.0040 | 95.2 | | | |
| 1 | 50% | 0.200 | 0.0208 | 36527 | 0.0187 | 89.9 | 90.33 | 0.45 | 0.49 |
| 2 | | 0.200 | 0.0208 | 36745 | 0.0188 | 90.3 | | | |
| 3 | | 0.200 | 0.0208 | 36874 | 0.0189 | 90.8 | | | |
| 1 | 100% | 0.400 | 0.0416 | 73015 | 0.0373 | 89.7 | 90.2 | 0.62 | 0.69 |
| 2 | | 0.400 | 0.0416 | 73211 | 0.0374 | 90.0 | | | |
| 3 | | 0.400 | 0.0416 | 73387 | 0.0378 | 90.9 | | | |
| 1 | 150% | 0.600 | 0.0623 | 110719 | 0.0566 | 90.9 | 91.06 | 0.20 | 0.22 |
| 2 | | 0.600 | 0.0623 | 110863 | 0.0567 | 91.0 | | | |
| 3 | | 0.600 | 0.0623 | 111175 | 0.0569 | 91.3 | | | |

**Standard deviation-SD, Relative standard deviation-RSD*

**Table 7: Recovery result of Desmethoxy Impurity.**

| Set | Level | ml Added | mg Added | Area | mg found | % Recovery | % Mean Recovery | SD* | RSD* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | LOQ | 0.044 | 0.0045 | 4059 | 0.0060 | 100.0 | 100.73 | 1.27 | 1.26 |
| 2 | | 0.044 | 0.0045 | 4120 | 0.00608 | 100.0 | | | |
| 3 | | 0.044 | 0.0045 | 4144 | 0.00612 | 102.2 | | | |
| 1 | 50% | 0.220 | 0.1011 | 69819 | 0.1031 | 100.5 | 100.73 | 0.251 | 0.24 |
| 2 | | 0.220 | 0.1011 | 69923 | 0.1033 | 100.7 | | | |
| 3 | | 0.220 | 0.1011 | 70147 | 0.1036 | 101.0 | | | |
| 1 | 100% | 0.440 | 0.2023 | 139634 | 0.2063 | 101.2 | 101.3 | 0.152 | 0.15 |
| 2 | | 0.440 | 0.2023 | 139890 | 0.2066 | 101.4 | | | |
| 3 | | 0.440 | 0.2023 | 139.986 | 0.2068 | 101.5 | | | |
| 1 | 150% | 0.660 | 0.3035 | 207504 | 0.3065 | 100.5 | 100.6 | 0.100 | 0.99 |
| 2 | | 0.660 | 0.3035 | 207739 | 0.3069 | 100.6 | | | |
| 3 | | 0.660 | 0.3035 | 207991 | 0.3072 | 100.7 | | | |

**Standard deviation-SD, Relative standard deviation-RSD.*

**PRECISION**

According to specification limit of impurities which is not more than 1.0% and 0.2%, amount of individual impurities will be 10 ppm and 2 ppm which is 1.0% and 0.2% of 1000 ppm (sample concentration). For repeatability sample containing all impurities injected at 100% level for six times and for intermediate precision sample containing all impurities at 50%, 100% and 150% level injected for intraday precision and interday precision and it is injected in 3 sets.

**Repeatability**

**Table 8: Repeatability result of Amino impurity.**

| Sr.no. | Area | Mean | SD* | RSD* |
|---|---|---|---|---|
| 1 | 139053 | 138524.7 | 1063.529 | 0.767754 |
| 2 | 138231 | | | |
| 3 | 138664 | | | |
| 4 | 139127 | | | |
| 5 | 136548 | | | |
| 6 | 139525 | | | |

**Standard deviation-SD, Relative standard deviation-RSD*

**Table 9: Repeatability result of Desmethoxy impurity.**

| Sr.no. | Area | Mean | SD* | RSD* |
|---|---|---|---|---|
| 1 | 80538 | 80753.5 | 1104.51 | 1.367755 |
| 2 | 80175 | | | |
| 3 | 79012 | | | |
| 4 | 81136 | | | |
| 5 | 82163 | | | |
| 6 | 81497 | | | |

**Standard deviation-SD, Relative standard deviation-RSD*

**Intermediate Precision**

**Intraday precision**

**Table 10: Intraday precision of Amino impurity.**

| 50% level | | | | | | |
|---|---|---|---|---|---|---|
| **Set** | **Level** | **Morning** | **Evening** | **Mean** | **SD*** | **RSD*** |
| 1 | 50 | 67138 | 67896 | 67517 | 535.9869 | 0.793855 |
| 2 | 50 | 69465 | 71137 | 70301 | 1182.283 | 1.681744 |
| 3 | 50 | 68971 | 70665 | 69818 | 1197.839 | 1.715659 |
| **100% level** | | | | | | |
| **Set** | **Level** | **Morning** | **Evening** | **Mean** | **SD** | **RSD** |
| 1 | 100 | 139053 | 140261 | 139657 | 854.185 | 0.611631 |
| 2 | 100 | 138231 | 139989 | 139110 | 1243.094 | 0.893605 |
| 3 | 100 | 136982 | 139544 | 138263 | 1811.608 | 1.310262 |
| **150% level** | | | | | | |
| **Set** | **Level** | **Morning** | **Evening** | **Mean** | **SD** | **RSD** |
| 1 | 150 | 207446 | 209613 | 208529.5 | 1532.3 | 0.734812 |
| 2 | 150 | 205369 | 202136 | 203752.5 | 2286.076 | 1.121987 |
| 3 | 150 | 209671 | 204764 | 207217.5 | 3469.773 | 1.674459 |

**Standard deviation-SD, Relative standard deviation-RSD*

**Table 11: Intraday precision of Desmethoxy impurity.**

| 50% level | | | | | | |
|---|---|---|---|---|---|---|
| **Set** | **Level** | **Morning** | **Evening** | **Mean** | **SD*** | **RSD*** |
| 1 | 50 | 38949 | 39451 | 39200 | 354.9676 | 0.90553 |
| 2 | 50 | 35436 | 36175 | 35805.5 | 522.5519 | 1.459418 |
| 3 | 50 | 39470 | 40492 | 39981 | 722.6631 | 1.807516 |
| **100% level** | | | | | | |
| **Set** | **Level** | **Morning** | **Evening** | **Mean** | **SD** | **RSD** |
| 1 | 100 | 80538 | 79932 | 80235 | 428.5067 | 0.534065 |
| 2 | 100 | 79568 | 81496 | 80532 | 1363.302 | 1.69287 |
| 3 | 100 | 82457 | 84791 | 83624 | 1650.387 | 1.973581 |
| **150% level** | | | | | | |
| **Set** | **Level** | **Morning** | **Evening** | **Mean** | **SD** | **RSD** |
| 1 | 150 | 122410 | 124113 | 123261.5 | 1204.203 | 0.97695 |
| 2 | 150 | 122654 | 124936 | 123795 | 1613.618 | 1.303459 |
| 3 | 150 | 124579 | 127894 | 126236.5 | 2344.059 | 1.856879 |

**Standard deviation-SD, Relative standard deviation-RSD*

**Interday precision**

**Table 12: Interday precision of Amino impurity.**

| 50% level | | | | | | |
|---|---|---|---|---|---|---|
| **Set** | **Level** | **Day 1** | **Day 2** | **Mean** | **SD*** | **RSD*** |
| 1 | 50 | 67138 | 68165 | 67651.5 | 726.1987 | 1.073441 |
| 2 | 50 | 69465 | 71293 | 70379 | 1292.591 | 1.836615 |
| 3 | 50 | 68971 | 70895 | 69933 | 1360.473 | 1.945396 |
| **100% level** | | | | | | |
| **Set** | **Level** | **Day 1** | **Day 2** | **Mean** | **SD** | **RSD** |
| 1 | 100 | 139053 | 141126 | 140089.5 | 1465.832 | 1.046354 |
| 2 | 100 | 138231 | 140640 | 139435.5 | 1703.42 | 1.221655 |
| 3 | 100 | 136982 | 139156 | 138069 | 1537.25 | 1.113393 |
| **150% level** | | | | | | |
| **Set** | **Level** | **Day 1** | **Day 2** | **Mean** | **SD** | **RSD** |
| 1 | 150 | 207446 | 209975 | 208710.5 | 1788.273 | 0.85682 |
| 2 | 150 | 205369 | 208361 | 206865 | 2115.663 | 1.022727 |
| 3 | 150 | 209671 | 205015 | 207343 | 3292.289 | 1.587847 |

**Standard deviation-SD, Relative standard deviation-RSD*

**Table 13: Interday precision of Desmethoxy impurity.**

| 50% level | | | | | | |
|---|---|---|---|---|---|---|
| **Set** | **Level** | **Day 1** | **Day 2** | **Mean** | **SD*** | **RSD*** |
| 1 | 50 | 38949 | 39973 | 39461 | 724.0773 | 1.834919 |
| 2 | 50 | 35436 | 36321 | 35878.5 | 625.7895 | 1.744191 |
| 3 | 50 | 39470 | 40597 | 40033.5 | 796.9093 | 1.990606 |
| **100% level** | | | | | | |
| **Set** | **Level** | **Day 1** | **Day 2** | **Mean** | **SD** | **RSD** |
| 1 | 100 | 80538 | 82467 | 81502.5 | 1364.009 | 1.673579 |
| 2 | 100 | 79568 | 81686 | 80627 | 1497.652 | 1.857507 |
| 3 | 100 | 82457 | 84807 | 83632 | 1661.701 | 1.98692 |
| **150% level** | | | | | | |
| **Set** | **Level** | **Day 1** | **Day 2** | **Mean** | **SD** | **RSD** |
| 1 | 150 | 122410 | 124205 | 123307.5 | 1269.257 | 1.029343 |
| 2 | 150 | 122654 | 126038 | 124346 | 2392.849 | 1.924348 |
| 3 | 150 | 124579 | 128149 | 126364 | 2524.371 | 1.997698 |

**Standard deviation-SD, Relative standard deviation-RSD*

**ROBUSTNESS**

According to robustness, there is a minor deliberate change made in chromatographic parameter with reference of flow rate and column temperature.

To observe robustness, 100% level solution is used.

**Change in Flow rate**

Inject the solution of 100% level of all impurities at a flow rate of 0.9 ml/min and 1.1 ml/min and Calculate RSD for all the responses of all impurities individually.

**Change in Column temperature**

Inject the solution of 100% level of all impurities at a column temperature of 25ºC and 35ºC and Calculate RSD for all the responses of all impurities individually.

**Table 14: Robustness result of Amino impurity.**

| Parameter | Change | Area | Mean | SD* | RSD* |
|---|---|---|---|---|---|
| Flow Rate(ml/min) | 0.9 | 129645 | 135809.6 | 2677.417 | 1.97145 |
| | 0.9 | 134944 | | | |
| | 0.9 | 136037 | | | |
| | 1.0 | 137153 | | | |
| | 1.0 | 136556 | | | |
| | 1.0 | 135570 | | | |
| | 1.1 | 136936 | | | |
| | 1.1 | 139664 | | | |
| | 1.1 | 135781 | | | |
| Column temperature | 25ºC | 138456 | 136779 | 2321.608 | 1.697343 |
| | 25ºC | 135475 | | | |
| | 25ºC | 137987 | | | |
| | 30ºC | 139451 | | | |
| | 30ºC | 136980 | | | |
| | 30ºC | 137583 | | | |
| | 35ºC | 134511 | | | |
| | 35ºC | 132145 | | | |
| | 35ºC | 138423 | | | |

**Standard deviation-SD, Relative standard deviation-RSD.*

**Table 15: Robustness result of Desmethoxy impurity.**

| Parameter | Change | Area | Mean | SD* | RSD* |
|---|---|---|---|---|---|
| Flow Rate(ml/min) | 0.9 | 80369 | 81248.78 | 1542.299 | 1.898242 |
| | 0.9 | 82374 | | | |
| | 0.9 | 84561 | | | |
| | 1.0 | 81008 | | | |
| | 1.0 | 80376 | | | |
| | 1.0 | 79163 | | | |
| | 1.1 | 81756 | | | |
| | 1.1 | 80487 | | | |
| | 1.1 | 81145 | | | |
| Column temperature | 25ºC | 82365 | 80572.22 | 1414.444 | 1.755498 |
| | 25ºC | 78751 | | | |
| | 25ºC | 81012 | | | |
| | 30ºC | 80136 | | | |
| | 30ºC | 79425 | | | |
| | 30ºC | 82456 | | | |
| | 35ºC | 81076 | | | |
| | 35ºC | 78693 | | | |
| | 35ºC | 81236 | | | |

**Standard deviation-SD, Relative standard deviation-RSD.*

## CONCLUSION

The proposed RP-HPLC method is linear, precise, accurate and robust for the determination of lacosamide and its related substance in parenteral dosage form and can be reliably adopted for routine quality control analysis of lacosamide and its related impurities in parenteral dosage form.

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