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# A Stability-Indicating Liquid Chromatographic Method for the Quantification of New Anti-Epileptic Drug Lacosamide and its Intermediates

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## ABSTRACT

A simple, selective, precise, and stability-indicating high performance liquid chromatography (HPLC) method has been established and validated for the determination of S (–) Enantiomer in Lacosamide drug substance. The chromatographic system used normal phase DAICEL Chiralcel OD-H column with UV-Vis detection at 210 nm. The mobile phase was a mixture of n–hexane–ethanol, 74:6 (%, v/v) and this mixture was transferred to isopropyl alcohol–trifluoroacetic acid in the ratio of 72:6:0.08 (%, v/v). The method is validated for its specificity, precision, accuracy, linearity and ruggedness. Regression analysis data for the calibration plots were indicative of good linear relationships between response and concentration over the range 0.0174µg $mL^{-1}$ – 5.398 µg $mL^{-1}$. The correlation coefficient, $r^2$, was 0.9994 and 0.9988. The value of slop and intercept of the calibration plot was 79403 and -16673. The limit of detection and quantitation were 0.087 ± 7.18 µg $mL^{-1}$ and 0.263 ± 3.68 µg $mL^{-1}$. Statistical analysis proved the method is repeatable, selective, and accurate for estimation of S (–) Enantiomer in Lacosamide drug substance and its intermediates. Because the method could effectively separate the drug from their possible impurities like dibenzyl urea, benzyl acetamide, desmethyl, diacetyl and methylbenzyl serine, it can be used as a stability indicating method.

## KEYWORDS

Normal phase, HPLC, UV-Vis detection, Chiralcel OD-H column, Limit of Quantitation, and Limit of Detection.

## INTRODUCTION

Lacosamide are indicated as adjunctive therapy for partial onset seizures in patients 17 years of age and older, the injectable is indicated as adjunctive therapy for partial-onset seizures when oral administration is temporarily not feasible in these patients1. Lacosamide was also submitted for review by the US Food and Drug Administration (FDA) in the treatment of diabetic neuropathic pain but has not been approved for this indication[1].

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Lacosamide was synthesized as a member of a family of functionalized amino acids, more specifically, analogues of the endogenous amino acid and NMDA-receptor modulator D-serine. The mechanism of action of Lacosamide has to be considered still not fully elucidated1. However, a dual mode of action is hypothesised: it selectively enhances slow inactivation of voltage-gated sodium channels (VGSC) and interacts with collapsin response mediator protein-2 (CRMP–2)[2-4], a protein mainly expressed in the central nervous system (CNS) and involved in neuronal differentiation and axonal outgrowth. Lacosamide showed an antiepileptic activity in different rodent seizure

models for generalized and complex partial-onset seizures and status epilepticus[5].

Lacosamide is not official in any pharmacopoeia; few liquid chromatography procedures have been reported for the determination of Lacosamide in Bulk and Pharmaceutical dosage forms[6–7]. However there are no publications concerning the analytical method of Lacosamide S (–) Enantiomer (Fig-2) in bulk drug and pharmaceutical dosage forms. The chemical name of S (–) Enantiomer is (S)–2–acetamido–N–benzyl–3–methoxypropanamide.

## MATERIAL AND METHOD

### Chemicals and Reagents

All chemicals were of the highest purity available. Trifluoroacetic acid (AR Grade), n-hexane (HPLC Grade), ethanol (AR Grade), isopropyl alcohol (HPLC Grade) were used as mobile phase chemicals and purchased from Ranchem. Lacosamide and Lacosamide S (–) Enantiomer was used as sample and standard, respectively.

### Chromatography

Chromatography was performed on a Shimadzu LC-2010$_{HT}$ with UV/ PDA detector and LC solution software. DIACEL Chiralcel OD-H, (250 x 4.6) mm, 5µm was used for separation and quantification of S (–) Enantiomer. Mettler Toledo XS 205 analytical balance were used for weighing of sample and standard preparation. The mobile phase was a mixture of n-hexane–ethanol in the ratio of 74:6 (%, v/v). This mixture was transferred to isopropyl alcohol and trifluoroacetic acid in the ratio of 72:6:0.08 (%, v/v). Chromatographic parameters were used as the detection was performed at 210 nm; the flow of mobile phase was 0.5 mL min$^{-1}$. The injection volume was 10 µL. The column oven temperature was 25°C. The total time required for chromatographic separation was 25 min.

### Method Development

Column chemistry, solvent selectivity (solvent type), solvent strength (volume fraction of organic solvent (s) in the mobile phase), additive strength, detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimised so the peak of S (-) Enantiomer did not interfere with those from the solvent and possible impurities. Other criteria, viz. time required for analysis, appropriate $k$ range ($1<k<10$) for elute peaks, sensitivity, solvent noise, were also considered. After each change of the mobile phase the column was re-equilibrated by passage of at least ten column volumes of the new mobile phase[8]. To investigate the appropriate wavelength for simultaneous determination of S (-) Enantiomer solution of the compound in the mobile phase were scanned by UV-visible spectrophotometry (Shimazdu, Japan model UV-1800) in the range 200-400nm. From the UV spectra, suitable wavelength choices considered for monitoring the S (–) Enantiomer was 210 nm. Solutions of S (-) Enantiomer in the mobile phase was also injected directly for HPLC analysis and the responses (peak area) was recorded at 210 nm. It was observed there was no interference from the mobile phase or base line disturbance at 210 nm. It was, therefore, concluded that 210 nm is the most appropriate wavelength for analysis of the S (–) Enantiomer with suitable sensitivity.

A variety of mobile phases were investigated to establish a suitable HPLC method for analysis of S (–) Enantiomer in Lacosamide. This included mixture of n-hexane–ethanol, 74:6 (%, v/v), n-hexane–ethanol–trifluoroacetic acid, 74:6:0.08 (%, v/v), n-hexane–ethanol–isopropyl alcohol–trifluoroacetic acid, 74:6:6:0.08 (%, v/v). The final mobile phase was a mixture of n-hexane–ethanol, 74:6 (%, v/v), this mixture was transferred to isopropyl alcohol–trifluoroacetic acid in the ratio of 72:6:0.08 (%, v/v). The suitability of the mobile phase and method was decided by study of the accuracy, precision, linearity, specificity, detection limit, quantitation limit and stability in analytical solution in accordance with USP and ICH guidelines[9-10].

## METHOD VALIDATION

The method was validated for accuracy, intra day and inter-day precision, linearity, specificity, detection limit, quantitation limit and stability in analytical solution, in accordance with ICH guidelines[9-10].

System suitability tests are an integral part of liquid chromatographic method. It is used to verify that the resolution of the chromatographic system is adequate for the analysis to be done[9-10]. The tests are based on the concept that the equipment, electronics, analytical operations and sample to be analyzed constitute an integral system that can be evaluated as such. System suitability was evaluated by replicate ($n$= 6) injections of the standard solution containing S (–) Enantiomer at 2.25 µg $mL^{-1}$. The *RSD* of retention time, peak area, number of theoretical plates, and USP tailing factor were within 1%, indicating the suitability of the system (Table I). The number of theoretical plates and the USP tailing factor were within the acceptance criteria of >2000 and ≤1.5, respectively, indicating good column efficiency and optimum mobile phase and optimum mobile phase composition[8].

Table 1: Results of system suitability

| Property [a] | S (–) Enantiomer | |
|---|---|---|
| | Mean [b] | RSD (%) |
| $t_R$ | 16.73 | 0.16 |
| *A* | 166223 | 0.86 |
| *T* | 1.25 | 0.52 |
| *N* | 6462 | 0.66 |

[a] $t_R$, retention time; A, peak area; T, tailing factor; N, number of theoretical plates;

[b] Mean from of six replicate injections ($n$ = 6).

### Specificity and selectivity

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, degradants, and matrix, etc.[9-10]. The specificity and selectivity of the proposed method was evaluated by estimating the amount of S (–) Enantiomer in the presence of Lacosamide, its possible impurities and Blank. The HPLC chromatograms was recorded for the spiked solution revealed no peaks within a retention time of S (–) Enantiomer. The study of the absence of Lacosamide, its possible impurities and blank showed that none of the peaks appears at the retention time of S (–) Enantiomer and it was concluded that the developed method is selective in relation to the S (–) Enantiomer of the final preparation. The chromatograms of Lacosamide, Lacosamide S (–) Enantiomer, Blank, Spiked and Blank samples using the proposed method is shown in [Fig-4 to Fig-8].

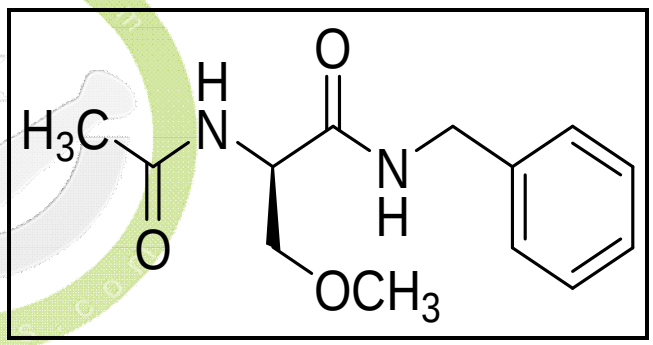



Figure 1: Structure of Lacosamide

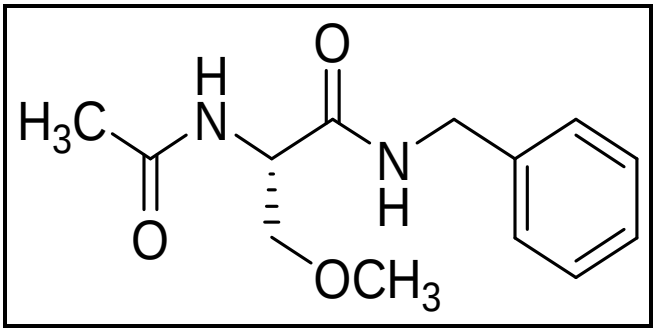



Figure 2: Structure of Lacosamide S (–) Enantiomer

### Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same

homogeneous sample under the prescribed conditions. Precision should be investigated using homogeneous, authentic samples[9-10]. In accordance with ICH recommendations precision was determined at two levels, i.e. repeatability and intermediate precision. Repeatability of sample application was determined as intraday variation whereas intermediate precision was determined by measuring inter-day variation from six preparation of spiking of S (–) Enantiomer in Lacosamide sample at target concentration level. Results from determination of repeatability and intermediate precision, as % *RSD*, are show in Table II. The low value of % *RSD* are indicative of the high repeatability of the method. Ruggedness of the method was established by comparing the results obtained on different days by different analyst on different instrument and HPLC column as % *RSD*.

Table 2: Results of measurement of intraday and inter-day precision ($n$ = 6)

| **Concn (µg mL$^{-1}$)** | **Repeatability (Intraday precision)** | | **Intermediate precision (Inter–day)** | | **Ruggedness (%) *RSD*** |
|---|---|---|---|---|---|
| | **Area** | **Mean (%) *RSD*** | **Area** | **Mean (%) *RSD*** | |
| 2.25 | 159861 | 0.37 | 168945 | 1.15 | 1.45 |
| | 158267 | | 170590 | | |
| | 158308 | | 169355 | | |
| | 158582 | | 173695 | | |
| | 159123 | | 170321 | | |
| | 158409 | | 167977 | | |

## Linearity

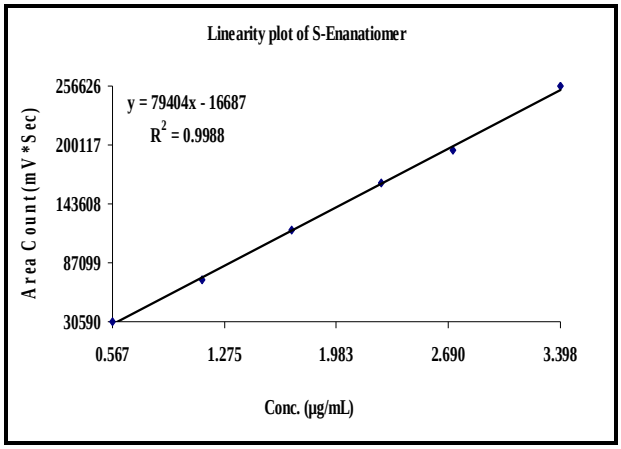
The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample[9-10]. The calibration graph was linear, i.e. the system adhered to Beer's law, over the range 25% to 300% of test concentration. The graph of area versus concentration is plotted. The correlation co-efficient (r), Y-intercept, slope of regression line, residual sum of squares are calculated and recorded in Table 3.

Table 3: Calibration plot data for S (–) Enantiomer

| **Concn (µg mL$^{-1}$)** | **Mean Area** |
|---|---|
| 0.567 | 30590 |
| 1.133 | 71231 |
| 1.698 | 118897 |
| 2.265 | 163162 |
| 2.718 | 194666 |
| 3.398 | 256626 |

Table 4: Linearity regression data for calibration plot for S (–) Enantiomer

| **Data** | **S (–) Enantiomer** |
|---|---|
| Linearity Range (µg mL$^{-1}$ ) | 0.567–3.398 |
| Regression equation | $y$ =79403x - 16673 |
| Correlation coefficient ($r$) | 0.9984 |
| Slope | 79403 |
| Intercept | -16673 |
| Residual sum of squares | 42496638 |




**Accuracy**

A study of recovery of S (–) Enantiomer in Lacosamide sample was established by spiking. Samples were prepared in triplicate by spiking of S (–) Enantiomer in test preparation at 50%, 100% and 150% of the target concentration level. The average % recovery of S (–) Enantiomer was calculated and given in Table 5. The recovery was found in the range of 88.33% to 95.51%, which indicated the accuracy of the method was adequate.

Table 5: Results from recovery studies ($n$ = 3)

| **Amount added (%)** | **Amount observed** | **Amount added** | **Amount present** | **Amount recovered** | **Mean (%) Recovery ± *RSD*** |
|---|---|---|---|---|---|
| 50 | 0.070 | 0.078 | 0.00 | 0.070 | 88.33 ± 0.82 |
| | 0.069 | 0.078 | 0.00 | 0.069 | |
| | 0.068 | 0.078 | 0.00 | 0.068 | |
| 100 | 0.150 | 0.156 | 0.00 | 0.150 | 95.51± 0.39 |
| | 0.149 | 0.157 | 0.00 | 0.149 | |
| | 0.149 | 0.157 | 0.00 | 0.149 | |
| | 0.150 | 0.157 | 0.00 | 0.150 | |
| | 0.149 | 0.156 | 0.00 | 0.149 | |
| | 0.149 | 0.156 | 0.00 | 0.149 | |
| 150 | 0.220 | 0.233 | 0.00 | 0.220 | 95.43± 1.67 |
| | 0.221 | 0.233 | 0.00 | 0.221 | |
| | 0.227 | 0.234 | 0.00 | 0.227 | |

**Limit of detection and quantification (LOQ and LOD)**

A study to establish the Limit of detection and Limit of Quantification of S (–) Enantiomer were conducted. Limit of detection and Limit of Quantification were established based on standard error and slop of linearity data. A series of solutions having S (–) Enantiomer were injected. Precision of S (–) Enantiomer at Limit of Quantitation and Limit of Detection was conducted. Six test preparations of S (–) Enantiomer at Limit of quantitation and Limit of Detection was prepared and injected into the HPLC system. The % RSD at LOQ and LOD level was calculated for S (–) Enantiomer and found 3.68 % for LOQ and 7.18 % for LOD.

**Stability in Solution**

The stability of S (–) Enantiomer spiked in sample in aqueous solution at a concentration of 2.28µg ml–1 was studied by storing the solution in a tightly capped volumetric flask at room temperature on a laboratory bench for 24 hours and found it was stable. The amount of the S (–) Enantiomer was checked at 0 hrs, 6hrs, 12hrs, 18hrs and 24hrs intervals.

Table 6: Summary of validation data

| Parameters | Concentration | Result |
|---|---|---|
| Linearity<br>Slope<br>Intercept | 0.567–3.398 µg $mL^{-1}$ | $R^2$ = 0.9984<br>76307<br>-9135 |
| Precision<br>(i) System Precision<br>% *RSD* (*n*=6) | 2.270 µg $mL^{-1}$ (standard) | 1.05 |
| (ii) Repeatability (Inter-day)<br>by recovery % *RSD* (n=6) | 0.15 % w/w | 0.37 |
| (iii) Intermediate precision (Intra-day) % *RSD* (n=6) | 0.15 % w/w | 1.15 |
| (iv)Ruggedness % *RSD* (n=6) | 0.15 % w/w | 1.45 |
| Accuracy<br>(Mean % Recovery, ± % *RSD*) | | |
| (*n* =3) at 50 % level | 0.075 % w/w | 88.33 ± 0.82 |
| (*n* =6) at 100 % level | 0.150 % w/w | 95.51 ± 0.39 |
| (*n* =3) at 150 % level | 0.225 % w/w | 95.43 ± 1.67 |
| (*n* = 12) Over all recovery | - | 93.69 ± 3.55 |
| Limit of Detection (% *RSD*) | 0.087 µg $mL^{-1}$ (0.0015 % w/w) | 7.18 |
| Limit of Quantitation<br>(% *RSD*) | 0.263 µg $mL^{-1}$ (0.005 % w/w) | 3.68 |
| Specificity | Specific | Specific |

*n* = number of determinations

## RESULTS AND DISCUSSION

During method development different approaches were tried. Presented method was found to be simple and sensitive with linearity in the concentration range of 0.567–3.398 µg $mL^{-1}$. Method is specific and indicated no interference in the S (–) Enantiomer peak, accuracy of method was established by recovery, the recovery values are within acceptable limits at different concentration levels and the data in table-1 indicated that the method is precise and rugged. Representative chromatogram is presented in Fig.-3 to Fig.-6 and the different values of validation data; linearity, precision, ruggedness, accuracy, limit of quantification and limit of detection are given in Table 6.

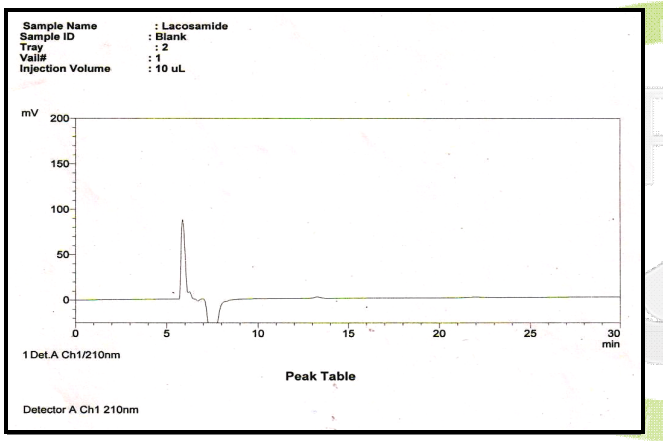
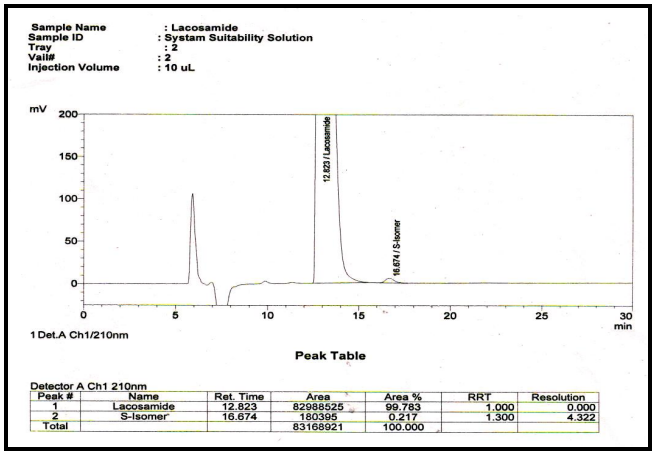
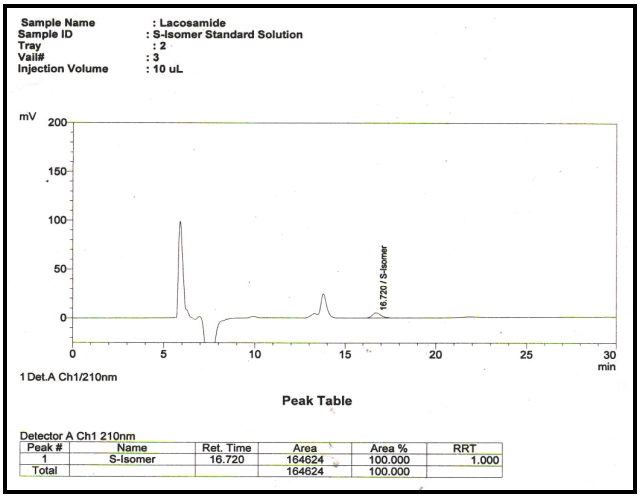



Figure 4: HPLC Chromatogram of Lacosamide Blank



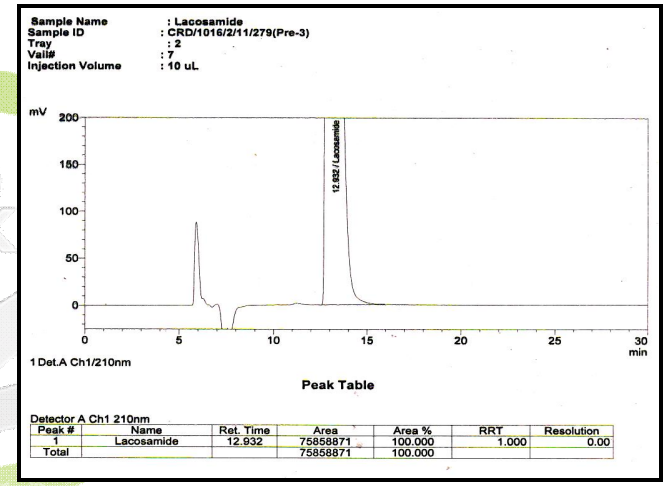

| Peak # | Name | Ret. Time | Area | Area % | RRT | Resolution |
|---|---|---|---|---|---|---|
| 1 | Lacosamide | 12.823 | 82988525 | 99.783 | 1.000 | 0.000 |
| 2 | S-Isomer | 16.674 | 180395 | 0.217 | 1.300 | 4.322 |
| Total | | | 83168921 | 100.000 | | |

Figure 5: HPLC Chromatogram of Lacosamide System suitability



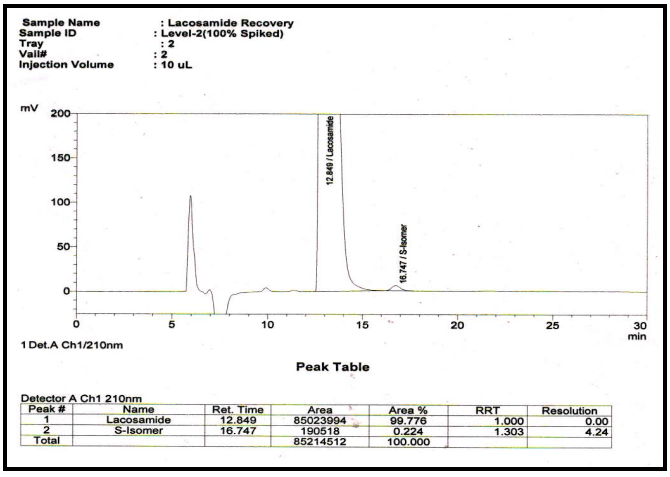

| Peak # | Name | Ret. Time | Area | Area % | RRT |
|---|---|---|---|---|---|
| 1 | S-Isomer | 16.720 | 164624 | 100.000 | 1.000 |
| Total | | | 164624 | 100.000 | |

Figure 6: HPLC Chromatogram of Lacosamide Standard




| Peak # | Name | Ret. Time | Area | Area % | RRT | Resolution |
|---|---|---|---|---|---|---|
| 1 | Lacosamide | 12.932 | 75858871 | 100.000 | 1.000 | 0.00 |
| Total | | | 75858871 | 100.000 | | |

Figure 7: HPLC Chromatogram of Lacosamide Sample




| Peak # | Name | Ret. Time | Area | Area % | RRT | Resolution |
|---|---|---|---|---|---|---|
| 1 | Lacosamide | 12.849 | 85023994 | 99.776 | 1.000 | 0.00 |
| 2 | S-Isomer | 16.747 | 190518 | 0.224 | 1.303 | 4.24 |
| Total | | | 85214512 | 100.000 | | |

Figure 8: HPLC Chromatogram of Lacosamide Spiked Sample

## CONCLUSION

This HPLC method is precise, specific, accurate, and stability indicating. Statistical analysis proved the method is repeatable and selective for the analysis of S (–) Enantiomer in Lacosamide. The method can be used to determine the purity of the drug obtained from different sources by detecting related impurities. It may be extended to determination of the possible impurities. Because the method separated the drug from its possible impurities, it can be used as a stability indicating method.

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