A Validated Chiral Liquid Chromatographic Method for the Enantiomeric Separation of Lacosamide Drug Product and its Dosage Forms

Charagondla K *
Analytical Research & Development, InvaGen Pharmaceuticals Inc., 7 Oser ave, Hauppauge, New York, 11788, USA

Abstract

A new and simple, rapid, selective isocratic normal phase liquid chromatographic (NP-LC) method was developed for the chiral purity of Lacosamide ((R)-2-acetamido-N-benzyl-3-methoxypropionamide) and its undesired S-enantiomer ((S)-2-acetamido-N-benzyl-3-methoxypropionamide). Superior resolution between Lacosamide and its S-enantiomer was achieved on Cellulose tris (3,5-Dichlorophenylcarbamate) immobilisation of polysaccharide derivatives based Chiralpak IC (25 mm x 4.6 mm, 5 µm) column using n-hexane : ethanol (85:15, v/v) as a mobile phase at 27°C column oven temperature. The USP resolution between the enantiomers was found more than five. Mobile phase flow was fixed at a rate of 1.0 mL/min and elution was monitored at 210 nm. The test concentration was 1000 µg/mL. Limit of detection and quantitation of S-enantiomer is 0.17 µg/mL and 0.48 µg/mL respectively. The percentage RSD of the peak area of six replicate injections of S-enantiomer at LOQ concentration was 5.8. The percentage recoveries of S-enantiomer from Lacosamide (LAC) were ranged from 105%-107%. The test solution and mobile phase were observed to be stable up to 48 h on bench top. The method was found to be selective, precise, linear, accurate and also robust. This method was successfully validated according to the International Conference Harmonization (ICH) guidelines.

Keywords: Lacosamide; Enantiomers; Chiral liquid chromatography; Validation; Specificity

Introduction

Epilepsy is a major neurological disorder that affects all populations [1]. Epilepsy describes the types of recurrent seizures produced by paroxysmal excessive neuronal disorders in the brain [2-3]. The mainstay of treatment has been the long-term and consistent administration of anticonvulsant drugs [4-6]. Unfortunately, current medications of treatment has been the long-term and consistent administration [1]. Epilepsy describes the types of recurrent seizures produced by Chiralpak IC CSP in the polar organic phase and normal-phase conditions. First, we selected a mobile phase capable of giving a baseline separation of LAC. The best analytical normal-phase condition was validated in terms of linearity, precision, accuracy, repeatability, ruggedness and robustness and limits of detection (LOD) and limit of quantification (LOQ) in order to quantify both enantiomers in active pharmaceutical ingredient and its drug dosage formulations.

This method can be able to separate all process impurities and degradants. It can also be able to separate its intermediate stages (penultimate stage) enantiomers. This method could be useful for reaction monitoring for synthesis of Lacosamide and its dosage forms. This method can be used for the routine regular analysis as well as the stability studies.

Experimental

Chemicals and reagents

N-Hexane was purchased from Merck (Worli, Mumbai, India), and Ethanol of AR grade (purity 99.9%) from Ching chaung Chemical, China. All the chemicals were used without further purification.

Received July 12, 2015; Accepted July 17, 2015; Published July 27, 2015

*Corresponding author: Charagondla Krishnaiah, Analytical Research & Development, InvaGen Pharmaceuticals Inc, 7 Oser ave, Hauppauge, New York, 11788, USA, Tel: 631-629-6242; Fax: 631-629-6242, E-mail: krishnaiah.charu@gmail.com


Copyright: © 2015 Charagondla K, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Apparatus

The liquid chromatography systems Waters alliance 2695 separation module with 2998 diode array detector (DAD) and Agilent (Wilmington, Delaware, USA) 1100 system equipped with photo DAD and a variable wavelength detector (VWD), were used for method development and method validations. The output signal was monitored and processed by using Waters (Milford, MA, USA) Empower-2 software. Chiralpak-IC (250 mm × 4.6 mm, 5 µm), Immobilized cellulose tris 3,5 dichlorophenylcarbamate, coated on 5 µm silica-gel support, was purchased from Daicel Chemical Industries (Tokyo, Japan).

Chromatographic conditions

Enantioseparation was achieved on a Chiralpack-IC column (250 mm × 4.6 mm, 5 µm) by using the mobile phase consisting of n-hexane: ethanol (85:15, v/v), delivered at a flow rate of 1.0 mL/min. The column oven temperature was maintained at 27°C and the wavelength was monitored at 210 nm. The injection volume was 10 µL. 2-Propanol (IPA) was used as diluent.

Preparation of test solution and control solution

Stock solutions of (R)-LAC, (S)-LAC, (R)-LAC-1 and (S)-LAC-1 (1000 µg/mL) were prepared individually by dissolving the appropriate amount in IPA and filled to the mark with IPA, and further diluting to required concentration with IPA.

Commercially available tablets containing 5 mg of LAC (Vimpact) (n=10) were pulverized with a pestle in a porcelain mortar. The tablet powder was taken into a 100 mL of volumetric flask, made up to the mark with IPA and the suspension was sonicated for 10-12 min and filtered through 0.45 µm membrane filters. The IPA solution was injected to LC system.

Weight percentage of each enantiomer (R, S) present in the sample was calculated by area normalization method because the other isomer was having the same response to that of R-LAC.

Method validation

The method was validated (in terms of sensitivity, linearity, accuracy, precision, solution stability, mobile phase stability, and robustness) in accordance with International Conference Harmonization (ICH) guidelines.

Specificity/Selectivity: The specificity/selectivity of the developed LC method was determined in the presence of its related impurities (Figure 1C and 1D), LAC enantiomers and degradation products. Forced degradation studies were also performed on LAC bulk drug samples to provide an indication of the stability-indicating property and specificity of the proposed method. The stress conditions employed for degradation study included light (1.2 million lx h/m²/240 h), heat (90°C/10 days), humidity (40°C/75% RH/10 days), acid hydrolysis (0.1 M HCl/10 mL/3 h at 70°C), base hydrolysis (0.025 M NaOH/10 mL/90 min at RT), and peroxide degradation (6% H₂O₂/10 mL /9 h at 70°C). About 5%-15% degradation was observed in forced degradation studies. The typical chromatograms of control sample and stressed samples are represented in Figures 2A-2C. Peak purity of stressed sample of R-LAC was checked by using photo diode array detector (DAD/PDA). Purity angle was found less than purity threshold in all stress samples and no interference was found from blank demonstrating the analyte peak homogeneity.

LOD and LOQ: LOD and LOQ represent the concentration of the analyte that would yield a signal/noise ratio (S/N) of 3 and 10, respectively. LOD and LOQ of (S)-LAC, (R)-LAC were determined by injecting a series of diluted solutions.

Precision and repeatability: Method precision was determined by measuring the repeatability (intra-day precision) and intermediate precision (inter-day precision) of retention times and peak areas for LAC enantiomers. The intra-day variability was performed by the
same analyst over one day, while inter-day precision was carried out by another independent analyst over three days. In order to determine the repeatability of the method, replicate injections (n=6) of 1.5 µg/mL of LAC enantiomers were carried out. The intermediate precision was evaluated over three days by performing six consecutive injections each day. Precision was reported as % of relative standard deviation (%RSD).

**Linearity:** The linearity evaluation was performed with the spiked standard solutions of (S)-enantiomer at the concentrations ranging from six concentration (n=6) levels from LOQ to 150% (LOQ, 50, 75, 100, 125 and 150%). Three injections (n=3) of each solution were made under the chromatographic conditions described above, using an injection volume of 10 µL. The calibration curve was drawn by plotting the peak areas of (S)-LAC and (R)-LAC against its corresponding concentration. The %RSD and Y-intercept of the calibration curve (linear regression equation) were computed.

**Accuracy:** Standard addition and recovery experiments were conducted to determine accuracy of other enantiomer method for

![Base degradation chromatogram](image1)

![Oxidative degradation chromatogram](image2)

![Acid degradation chromatogram](image3)

**Figure 2:** The degradation/specificity chromatograms of LAC.
quantification of the impurities in bulk drug samples and dosage forms. The study was carried out in triplicate at 0.375, 0.75 and 1.125 µg/mL concentrations, whereas the analyte concentration was 1000 µg/mL. The percentage recoveries of (S) and (R)-LAC were calculated.

Solution stability and mobile phase stability: The solution stability of LAC and its enantiomers in this method was carried out by leaving spiked sample solution in tightly capped volumetric flask at room temperature (bench top) for 48 h. Contents of other enantiomer were determined for every 6 h interval. Mobile phase was also carried out for 48 h by injecting the freshly prepared sample solutions for every 6 h interval. Contents of other enantiomer were checked in the test solutions. Mobile phase prepared was kept constant during the study period.

Robustness: To determine the robustness of the developed method, the chromatographic conditions were deliberately altered and verified including the system suitability criteria. Establish the selectivity factor in all the robustness studies for enantiomer impurity and compare with regular experiment. The flow rate of mobile phase was 1.0 mL/min, to study the effect of flow rate on the peak USP tailing and USP resolution between enantiomers, flow rate was altered by 0.2 units, i.e, from 0.8 to 1.2 mL/min. The effect of composition of ethanol ratio on peak USP resolution and tailing of LAC were studied by varying ± 10% ethanol to 1.2 mL/min. The effect of composion of ethanol and temperature on peak USP tailing and USP resolution was studied by varying ± 10% ethanol and temperature of 27°C ± 5°C (22°C, 27°C and 32°C).

Results
Method development
The aim of this work was to separate the enantiomers for the accurate quantification of (S)-enantiomer. Racemic mixture solution of 1 mg/mL prepared in IPA was used in the method development. To develop a rugged and suitable LC method for the separation of LAC enantiomers, different mobile phases and stationary phases were employed. Initial development trials were carried out on the chiralpak AD-H column with the mobile phase combination of n-hexane and ethanol in the ratio of 90:10 (v/v). The peaks were retained very early and selectivity could be very poor. With further increasing the ratio of ethanol by ten percent volume, selectivity was remains same, but the peak shape was broad with tailing. After that introduced chiralcel OD-H and chiralpak-AS columns with different combinations of mobile phases but individual pair resolutions were observed with tailing but the selectivity was poor (Figure 3A and 3B), with merged (S)-LAC and (S)-Imp-1 peaks. Apply above mobile phase combination to phenomenex Lux Cellulose-2 column and get individual separation for each enantiomers, but (R)-LAC and (R)-Imp-1 were merged Figures 3C and 3D. Finally introduce chiralpak-IC immobilized stationary phase initially with n-hexane and ethanol in the ratio of 50:50 (v/v), (R)-LAC and (S)-Imp-1 peaks. Apply above mobile phase combination to Chiralpak IC CSP, has been recently launched by Daicel Chemical Industries Ltd. The Chiralpak IC used cellulose tris (3,5- dichlorophenylcarbamate) immobilised onto silica 5 micron particles as a chiral selector. In the first part of our study, the resolving ability of the Chiralpak IC CSP towards LAC was investigated in the nonpolar organic mode using pure n-Hexane, n-Hexane and Ethanol with a ratio of 85:15 (v/v). The resolving power of the IC CSP was sufficiently high to achieve a baseline enantioseparation in each of the used conditions. The best resolution value was achieved by using the n-Hexane and Ethanol with a ratio of 85:15 (v/v) eluent with enantioselectivity factor (k) and resolution factor (Rs) values of 1.3 and 5.7, respectively (Figure 3I).

Chemoselective analysis
A critical aspect in the development of an analytical method to determine the enantiomeric purity of a chiral drug is the presence of impurities from the processes of synthesis, degradation or manufacturing either in the drug substance or in the finished product. The main organic impurities of LAC process in the manufacturing process are checked and found no interference from impurities. Stability studies, chemical development studies, routine batch analyses and quality control of the commercial product require a chemo- and enantioselective method capable of discriminating the enantiomeric API (Active Pharmaceutical Ingredient) from the related substances. Consequently, we turned our attention to examine the chemical selectivity of the Chiralpak IC using the n-Hexane- Ethanol (absolute 85:15, v/v) as a mobile phase. In these conditions, the LAC enantiomers were well separated and there was no overlapping with potential organic impurities. From Figure 3H, it can be seen that all the process and penultimate impurities were resolved with a k-value ranging from 1.1 to 1.3. The graphical approach for mobile phase composition verses USP resolutions (Figure 4).

Validation of the method
System suitability: The system suitability solution is prepared as mentioned in the preparation of the test solution and the control solution, to obtain a final concentration of LAC 1000 µg/mL spiked with 1.5 µg/mL of LAC enantiomer. The resolution is not less than 3.0 (Figure 3I).

LOD and LOQ: LOD and LOQ were established from S/N ratio methodology. LOD and LOQ were estimated to be 15 and 48 µg/mL for each enantiomer of (S)-LAC with good precision at LOD. Precision at LOQ was established by calculating %RSD for replicate injections (n=6) of reference standard prepared at LOQ level. The determined LOD, LOQ limit of quantification and precision at LOQ values are reported in Table 1.
3A: Spiked chromatogram (Chiralcel-OD-H).

3B: Spiked chromatogram (Chiralpak-AS).

3C: (R), (S)- Imp-1 spiked chromatogram (Lux cellulose-2).

3D: (R), (S)- LAC spiked chromatogram (Lux cellulose-2).
3E: Spiked chromatogram (Chiralpak-IC MP: n-Hexane: Ethanol (50:50 v/v)).

3F: Spiked chromatogram (Chiralpak-IC MP: n-Hexane: Ethanol (70:30 v/v)).

3G: Spiked chromatogram (Chiralpak-IC MP: n-Hexane: Ethanol (90:10 v/v)).

3H: Spiked chromatogram (Chiralpak-IC MP: n-Hexane: Ethanol (85:15 v/v)).
Figure 3: Blend Chromatogram of LAC and its impurities.

Figure 4: Graphical representation for mobile phase composition versus USP resolution.

Precision/Repeatability/Method: Method reproducibility was determined by measuring repeatability, precision, and intermediate precision (between-day precision). Repeatability of the method was determined by calculating %RSD for the enantiomer area from replicate injections (n=6) of the system suitability solution. The %w/w values of the enantiomer for six different preparations of the system suitability solution were calculated. Method precision was determined by calculating %RSD for these %w/w values Table 1.

Intermediate precision was determined by performing method precision by a different analyst on a different day. The results in Table 2 show that method reproducibility was good.

Linearity: The linearity of the HPLC method was evaluated by injecting standard concentrations of racemic samples with a concentration ranging from LOQ to 150%, i.e, 0.48 µg/mL and 2.25 µg/mL for single enantiomer. The peak area response of the both enantiomers was plotted versus the nominal concentration of the enantiomer. The linearity was evaluated by linear regression analysis, which was calculated by least squares regression method. The obtained calibration curve showed correlation coefficient greater than 0.9994 for (S)-LAC. The following regression equation was obtained y=251717x - 73.289 (R²=0.9988) where y is the peak area of (S)-LAC and x is the concentration. For (R)-LAC the following regression equation was obtained y=244532x + 398592 (R²=0.9999).

Accuracy: Accuracy for the determination of enantiomeric composition was determined by preparing three drug substance samples at 50%, 100% and 150% of the target concentration (0.075%,
0.15% and 0.225%). Apparent recovery was ranged from 105% to 107%. Overall percent recovery was 106 (RSD%-0.8) for the enantiomer (S)-LAC Table 3.

**Solution stability:** Stability of the sample in the diluent was evaluated by injecting the freshly prepared spiked test solution every 6 h up to 48 h, at room temperature bench top solution. Physical appearance, for example, color of the solution did not change; no extra peaks appeared; and the cumulative relative standard deviation for the peak area of LAC enantiomer was less than 2.0%. Peak purity obtained from the photodiode array detector for the LAC enantiomer peak, showed that the peak was pure (purity angle is less than purity threshold). The results indicated that the solutions were stable up to 48 h at room temperature bench top solution.

**Mobile phase stability:** Mobile phase was also carried out for 48 h by injecting the freshly prepared spiked sample solutions for every 6 h interval. Physical appearance, no turbidity/haziness were observed mobile phase during analysis, Mobile phase prepared was kept constant during the study period with no extra peaks appeared, and the cumulative relative standard deviation for the peak area of LAC enantiomer was less than 2.0%. Peak purity obtained from the photodiode array detector for the LAC enantiomer peak, showed that the peak was pure (purity angle was less than purity threshold). The results indicated that the solutions were stable up to 48 h at room temperature bench top solution.

**Robustness:** The robustness of a method is the ability of the method to remain unaffected by small but deliberate changes in parameters such as flow rate, mobile phase composition, and column oven temperature. The effect of resolution for the system suitability solution was monitored in this study. The flow rate of the mobile phase is 1.0 mL/min and was varied to 0.8 mL/min and 1.2 mL/min. The effect of change in percent organic, i.e., ethanol, (9:1; 8:2 v/v) ratio was studied by varying its concentration to -10 and +10% absolute, i.e., n-hexane: ethanol (90:10, v/v); n-hexane: ethanol (80:20, v/v). The column temperature is ambient (oven temperature maintained at 27°C) and was varied to 32°C and 22°C. The effects of each of these parameters were studied while the rest of the parameters were kept constant, as mentioned in an above section. The resolution remained unaffected by these small changes as shown in Table 5, thus proving the method to remain unaffected by small but deliberate changes in parameters.

**Discussion**

A simple and new analytical chiral method has been developed for Lacosamide intermediate stages, Lacosamide drug product and its dosage forms. This method can be able to separate all process impurities. This method has been validated as per ICH and proved for specific. This method could be useful for reaction monitoring for synthesis of Lacosamide and its dosage forms.

**Conclusion**

In conclusion, a simple, sensitive, chemo- and enantio selective LC method for the quantitative determination of the enantiomers of LAC was developed and validated using the immobilised-type LC method for the quantitative determination of the enantiomers of Lacosamide and its dosage forms. This method could be useful for reaction monitoring for synthesis of Lacosamide and its dosage forms. This method can be able to separate all process impurities. This method has been validated as per ICH and proved for specific. This method could be useful for reaction monitoring for synthesis of Lacosamide and its dosage forms. This method has been validated as per ICH and proved for specific. This method could be useful for reaction monitoring for synthesis of Lacosamide and its dosage forms. This method can be used for the routine analysis as well as the stability studies to evaluate (R) →(S), (S) →(R) isomerisation in stage compounds. Chiralpak IC CSP in normal-phase conditions. The proposed method meets the requirements of the all guidelines and is a reliable way to determine the enantiomeric purity of LAC penultimate stages and LAC in bulk drugs and pharmaceutical dosage forms. This method can be used for the routine analysis as well as the stability studies to evaluate (R) →(S), (S) →(R) isomerisation in pharmaceutical quality control. Chiralpak IC CSP in normal-phase conditions. The proposed method meets the requirements of the all guidelines and is a reliable way to determine the enantiomeric purity of LAC penultimate stages and LAC in bulk drugs and pharmaceutical dosage forms. This method can be used for the routine analysis as well as the stability studies to evaluate (R) →(S), (S) →(R) isomerisation in pharmaceutical quality control.

**References**