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Research Article

REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-ULTRAVIOLET-BASED APPROACH FOR METHOD DEVELOPMENT AND VALIDATION OF LACOSAMIDE ESTIMATION IN HUMAN SERUM

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ABSTRACT

Objective: The main aim of the study was to develop and validate a simple, accurate, and rapid isocratic reverse phase high-performance liquid chromatographic method with UV detection for the determination of lacosamide, an antiepileptic agent, in human serum.

Methods: Chromatographic separation was performed using a reverse phase chromatographic column (Zorbax SB-C18, 5 µm 4.6×250 mm) with a mobile phase being a mixture of potassium dihydrogen phosphate buffer (pH adjusted to 3.0 with orthophosphoric acid) and acetonitrile (ratio of 83:17 v/v) at a flow rate of 1.2 mL/min. UV detection was carried out at 210 nm and the sample temperature was maintained at 4°C.

Results: Linear calibration curves in the range of 1.012–40.894 µg/ml gave a correlation coefficient of 0.9988. The intra-day (n=6) and inter-day (n=18) precision (expressed as relative standard deviation) were in the range of 0.79–2.485% and from 0.99 to 3.21%, respectively. The retention time (in minutes) of lacosamide and internal standard was approximately 8.785±0.19 and 3.985±0.77, respectively, with no matrix interference. The method was validated for system suitability, specificity, precision, accuracy, robustness, linearity, limit of detection, and limit of quantification following the International Conference on Harmonization guidelines. The method was further validated using sera of epileptic patients consuming lacosamide, and it was observed that the results matched with the patients' clinical response.

Conclusion: Our method developed to estimate serum lacosamide level is simple, cost-effective, and reliable for therapeutic drug monitoring.

Keywords: RP-HPLC, Lacosamide, Method development, Validation, Human serum.

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INTRODUCTION

Lacosamide is a functionalized amino acid that is currently licensed as an antiepileptic drug for use either singly or as adjunctive therapy in the treatment of partial (focal) onset seizures in adults and children 4 years of age or older in various countries, including India [1]. Lacosamide is marketed in India under different brand names such as Ictrol, Lacasa, Lacopsy, Lacosam, and Lacoset both as oral and injectable formulations [2].

Lacosamide is hypothesized to have a novel dual mechanism of action. It selectively enhances sodium channel inactivation and is involved in the modulation of collapsin response mediator protein-2. It works by reducing the activity of sodium channels that allow electrical impulses to be transmitted between nerve cells. This prevents abnormal electrical activity in the brain, which, in turn, lowers the chance of an epileptic seizure [3,4]. Lacosamide has excellent oral absorption, high bioavailability, negligible protein binding, and is excreted mainly in the urine. Lacosamide does not induce or inhibit cytochrome P450 enzymes or known drug transporter systems and, because it has multiple elimination pathways, it has no clinically relevant interactions with commonly prescribed medications [5].

Therapeutic drug monitoring (TDM) is a useful tool in the clinical management of epilepsy with antiepileptic drugs (AEDs) as it helps optimize seizure control, monitor inter-individual variability, drug-drug and drug-food interactions, alterations due to impaired organ function, genetic factors, pregnancy, treatment in specific age groups (children and the elderly), and adherence, especially for those on long-term medication [6].

Literature survey reveals that there are very few liquid chromatography (LC) procedures that have been reported for the determination of lacosamide in human serum [7-12].

In this study, we describe a simple validated reverse phase high-performance liquid chromatographic (RP-HPLC)-ultraviolet (UV) method that quantitatively determines serum lacosamide levels, which is suitable for application in a TDM setting. Liquid-liquid phase extraction method was used to prepare the serum samples prior to lacosamide estimation in the analytic RP-HPLC-UV system. Caffeine was used as the internal standard (IS) for quality control purposes.

METHODS

Method development

Instrumentation and chromatographic conditions

A high-performance LC system manufactured by Thermo Scientific consisting of a UV detector, quaternary solvent manager, sample manager, and column heating compartment was used for the determination of lacosamide. The software used in the HPLC system was Chromquest software. Kern analytical balance was used for weighing, a digital pH meter was used for adjusting the buffer pH, a Citizon ultrasonic cleaner was used to dissolve the standards, and centrifugation was done using a Hettich centrifuge machine. A reverse phase chromatographic column [Zorbax SB–C18, 5 µm 4.6×250 mm] was used as the stationary phase for chromatographic separation. The flow rate of the mobile phase was set to 1.2 ml/min. The autosampler temperature was kept at 20°C and the temperature of the column was maintained at 40°C. The detection wavelength was set at 210 nm.

Chemicals and reagents

The reference standards of Lacosamide and Caffeine (IS) were purchased from Sigma-Aldrich (Mumbai, India). Chemicals such as acetonitrile (HPLC grade; Merck), methanol (HPLC grade; Merck), orthophosphoric acid, and potassium dihydrogen phosphate were purchased from Merck (Darmstadt, Germany). Human blank serum used for the development and validation of the procedure was obtained from healthy volunteers.

Preparation of mobile phase

1.37 g of potassium dihydrogen phosphate was dissolved in 1000 mL of double distilled water, and the pH was adjusted to 3.0 using orthophosphoric acid. This solution was then sonicated to degas the buffer. 830 mL of buffer and 170 mL of acetonitrile were transferred in to a 1000 mL mobile phase bottle, mixed and sonicated up to 15 min to degas the mobile phase, following which the solution was filtered through 0.45 μm nylon membrane filter under vacuum.

Preparation of standard stock solutions and IS working standard solutions

10 mg of lacosamide reference standard was accurately weighed and transferred into a 10 ml volumetric flask containing 7 ml acetonitrile. The mixture was sonicated for 10 min to dissolve the drug completely. The volume was then adjusted with acetonitrile to get a stock solution of 1000 μg/ml. Aqueous trials of lacosamide were prepared by making the required dilutions of 40 μg, 30 μg, 20 μg, 10 μg, 5 μg, 2.5 μg, and 1 μg, respectively. The stock solution of the IS was prepared by dissolving 10 mg of 8-(3-Chlorostyryl) caffeine in 10 mL of methanol. Working solutions of the IS were prepared by a one-step dilution of the stock solution with methanol to achieve a concentration of 0.5 mg/ml.

Preparation of serum sample

200 μl of sample (standard/patient's serum) was transferred to a 1.5 ml Eppendorf tube. 5 μl of 0.5 mg/ml IS solution and 300 μl acetonitrile were added. The mixture is then vortexed for 10 min using a scientific vortex at maximal intensity and consequently centrifuged for 10 min in an Eppendorf centrifuge at 12,000 rpm at 21°C. Subsequently, 100 μl of the supernatant was transferred to an HPLC glass vial. The vials were then loaded onto the autosampler and 10 μl injected into the HPLC system.

Method validation parameters

Method validation was carried out according to the International Conference on Harmonization guidelines using the following parameters: System suitability, robustness, specificity, linearity, range, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) [13].

System suitability testing

Plate count (N), tailing factor (T), RT, and reproducibility (% relative standard deviation [%RSD]) were determined from replicate injections of a standard (an analyte peak and an IS, related compound, excipients, and/or impurity) compared against method specifications. System suitability was conducted using standard preparation and evaluated by injecting five replicate injections as the %RSD specification is below 2.0%.

Robustness

Robustness was performed by changing the pH of the buffer, column temperature, flow rate of the mobile phase, and wavelength. Robustness of the method was carried out in triplicates and the mean %RSD was calculated.

Specificity

To ensure that there was no interference between the RT of lacosamide with that of the IS, the diluents used and/or the degradation products that may form during the extraction process, the specificity of the method was determined. Specificity of the method was assessed by comparing chromatograms of blank serum samples and spiked serum samples within the concentration range from 1 μg to 40 μg, the diluents used, the IS, and the reference standard.

Linearity and range

Linearity of lacosamide was estimated in the range of 5–200% of the specification limit. A linear relationship between peak area and concentration was evaluated. The area response for each level was recorded and the slope, intercept, and correlation coefficient were calculated.

Precision

Method and system precision were determined by initially preparing a sample solution of lacosamide injection. Repeatability was then carried out using six replicates of the same concentration (10 μg/mL). Precision of the method was assessed by executing replicate analysis of the seven quality control samples (1, 2.5, 5, 10, 20, 30, and 40, with each concentration prepared in three replicates) against the calibration curve. Intra-day precision was tested by performing the analytical run twice daily at 3-hourly intervals while and inter-day precision was tested by performing the analytical run daily for 6 consecutive days. Precision was calculated using the percentage RSD for repeated measurements.

Accuracy

Accuracy of the method was determined by measuring the recovery of lacosamide sample solutions at different concentration levels ranging from 5% to 200%.

Detection and quantification limits

The LOD and the LOQ were evaluated with the help of a calibration curve of three samples with low concentration. The LOD was calculated using the formula 3.0 σ/S phenomena of the calibration curve, and the LOQ was calculated using the formula 10 σ/S phenomena of the calibration curve for the LOD and quantification, respectively, where σ is the standard deviation of the y-intercepts and S is the slope of the calibration curve.

RESULTS

Method development

Different mobile phases were employed to achieve the best separation and resolution when developing a suitable and robust LC method for the determination of Lacosamide. Among the different combinations of mobile phases tested, the mobile phase consisting of potassium dihydrogen phosphate buffer (pH adjusted to 3.0 with orthophosphoric acid): Acetonitrile in a ratio of 83:17 v/v gave the best resolution with sharp peaks and a RT of 8.785±0.19 for lacosamide and 3.985±0.77 for the IS, respectively, with no matrix interference. A Zorbax SB-C18, 5 μm 4.6×250 mm was used keeping the column temperature at 40°C. UV detection was performed at 210 nm, and the sample temperature was maintained at 5°C.

Method validation

System suitability

The %RSD from five replicate injections of the diluted standard preparation was 0.91%. The system suitability data are given in Table 1.

Robustness

The robustness of the method was assessed by injecting the system suitability solution in different conditions, namely, change in the pH of buffer solution, change in column temperature, flow rate, and wavelength. The results showed that the mobile phase was at its optimal at pH 3.0 with a column temperature of 40°C, flow rate at 1.2 ml/min, and at a wavelength of 210 nm. The results obtained are summarized in Table 2.

Specificity

The specificity of the method is illustrated in Fig. 1 wherein complete separation of the peaks of interest at RT was obtained using blank serum and serum spiked with lacosamide. In addition, there was

no interference at the RT of lacosamide and the IS (Caffeine). Lacosamide and the IS were efficiently separated and detected at the RT of 8.785±0.19 min and 3.985±0.77 min, respectively, indicating the specificity of the method.

Table 1: System suitability results of lacosamide

Injection volume (µl)	Retention time (min)	Tailing factor (NMT 2.0)	Plate count (N) (NLT 2000)
5	8.768	1.04	5379
10	8.785	1.15	5402
20	8.77	1.31	5456
30	8.79	1.47	5483
40	8.78	1.59	5491
Mean	8.78		5442.2
SD	0.0098		49.61
%RSD	0.11		0.91

RSD: Relative standard deviation

Table 2: Robustness results for lacosamide

Condition	%RSD	Theoretical plates (NLT: 2000)
Normal Condition (as such condition)	0.91	5442
Change in buffer pH 2.50	1.04	5321
Change in buffer pH 3.00	0.67	5468
Column temperature 40°C	0.78	5517
Column temperature 50°C	1.02	5643
Flow rate 1.00 ml/min	1.09	5589
Flow rate 1.20 ml/min	0.91	5442
Wavelength 205 nm	1.12	5326
Wavelength 210 nm	0.88	5439
Wavelength 215 nm	1.05	5547

Spectral analysis and evaluation of the RT of lacosamide and IS was done using the Chromquest software. Lacosamide gave a well-separated peak at a RT of 8.785 min. Caffeine, the IS, also gave a well-separated peak at a RT of 3.985 without interfering with lacosamide at a wavelength of 210 nm.

Linearity

Linearity with the lacosamide standard in the range of 5–200% of the specification limit was demonstrated. The calibration curve was found to be linear with a coefficient of correlation of 0.9988. The linearity curve of lacosamide is shown in Fig. 2.

Precision

Precision of the method was validated by assaying six lacosamide samples and calculating the RSD. A sample solution of lacosamide of a single concentration (10 µg/mL) was prepared and repeatability of the standard application was tested using six replicates of the same concentration. The %RSD for method precision was 0.67 (Table 3). Repeatability of the standard application was also carried out using six replicates of the same standard concentration (10 µg/mL) for system precision and the %RSD obtained was 0.93 (Table 4). Precision of the method was also assessed by executing replicate analysis of the seven quality control samples for both intra-day and inter-day repeatability. The RSD ranged from 0.79 to 2.37 and from 0.99 to 3.21 for intra- and inter-day analyses, respectively (Table 5).

Accuracy

Estimation of accuracy of the method showed that the mean percent (%) recovery was more than 100.07% and <107.35% at each level of

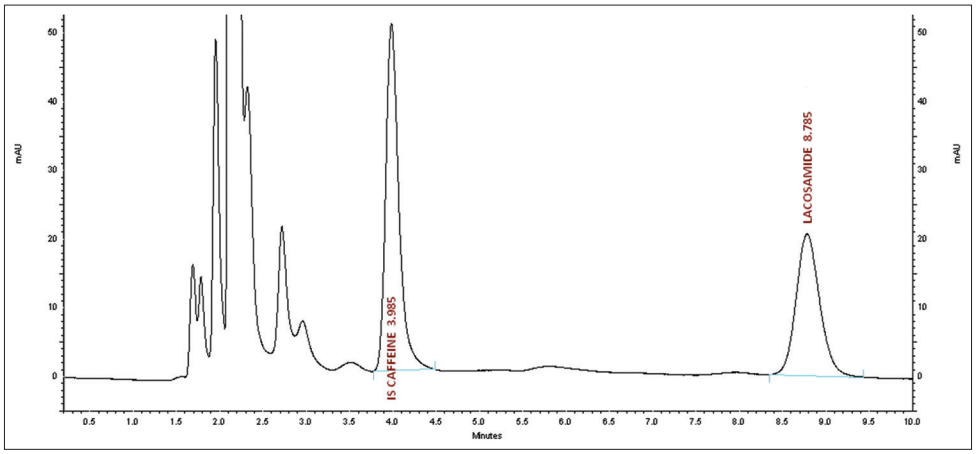

Fig. 1: Spectral analysis and evaluation of the retention time of lacosamide and internal standard

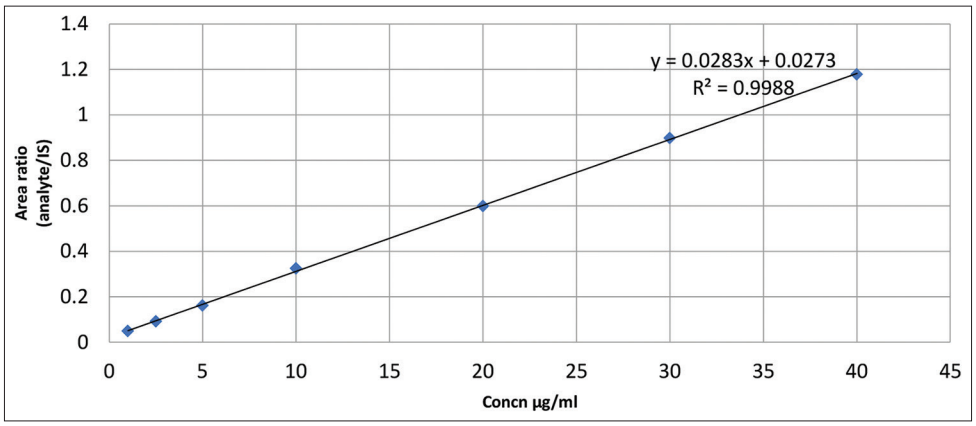

Fig. 2: Calibration curve of lacosamide in serum

the concentration range tested from 5% to 200% confirming that the method was accurate. The accuracy results are given Table 6.

Detection and quantification limits

The lowest LOD and the lowest LOQ were found to be 1.21 µg/ml and 3.67 µg/ml, respectively, which were well within the therapeutic range.

Application

The above validated RP-HPLC method was applied to perform drug analysis of patients receiving oral doses of lacosamide. The established therapeutic reference range for lacosamide is 3–10 µg/ml [14].

Table 3: Method precision of lacosamide

Injections	Area
1	4301427
2	4311434
3	4296991
4	4242538
5	4279649
6	4324512
Mean	4292758.5
SD	28789.14
%RSD	0.67

RSD: Relative standard deviation

Table 4: System precision of lacosamide

Injections	Area
1	4615880
2	4529651
3	4601246
4	4619723
5	4539645
6	4624562
Mean	4588451.17
SD	42514.63
%RSD	0.93

RSD: Relative standard deviation

Table 5: Intra-day and Inter-day precision of Lacosamide in serum

Concentration (µg/ml)	Measured concentration±S.D, %RSD	
	Intra-day (n=6)	Inter-day (n=6)
1	0.98±0.018; 1.93	1.02±0.022; 2.12
2.5	2.53±0.024; 0.94	2.48±0.025; 0.99
5	5.19±0.129; 2.49	4.90±0.124; 2.53
10	9.90±0.099; 1.00	10.25±0.108; 1.05
20	19.81±0.224; 1.13	20.70±0.546; 2.64
30	31.06±0.245; 0.79	29.53±0.760; 2.57
40	41.59±0.986; 2.37	38.99±1.25; 3.21

Table 6: Accuracy results of lacosamide in serum

Spike level	Lacosamide concentration	Analyte/ IS Ratio	Found concentration	Recovery %
5%	1	0.05	1.01	101.27
12.50%	2.5	0.09	2.54	101.63
25%	5	0.16	5.00	100.07
50%	10	0.32	10.74	107.35
100%	20	0.59	20.41	102.08
150%	30	0.89	30.98	103.29
200%	40	1.17	40.89	102.23
Mean (n=7)				102.56
SD				2.33
Overall %RSD				2.27

Lacosamide estimation in 60 epileptic patient samples showed that values of 38 patient samples were within the therapeutic range while those of 22 patients were below the therapeutic range. The daily lacosamide dose of patients with serum lacosamide concentrations within the therapeutic range ranged between 100 and 300 mg per day and the drug levels correlated with their adherence to therapy and seizure frequency which was low. Fourteen of the 22 patients in whom the drug level was below the therapeutic range were consuming low doses of lacosamide (50–100 mg/day) and had history of poor compliance with missed doses reported. The remaining eight patients with low drug levels had no clinical complaints with respect to seizure frequency.

DISCUSSION AND CONCLUSION

Therapeutic drug concentration monitoring TDM is carried out for a number of antiepileptic drugs which helps establish optimal therapy regimens for individual patients. This approach is a valuable tool for better management of drug dosage in patients who do not respond to a particular dose. TDM has the potential to assess noncompliance and to study pharmacokinetic variations that occur between individuals.

Lacosamide is one of the newer AEDs that has been approved as add-on treatment alongside other AEDs to treat focal seizures in adults and children more than 4 years of age especially for drug-resistant epilepsy [15]. Lacosamide has a favorable pharmacokinetic profile in terms of its availability as both oral and intravenous formulations, linear, and dose-proportional pharmacokinetics, rapid, and complete absorption that is unaffected by food, minimal protein binding and, unlike various AEDs, and low potential for clinically relevant drug–drug interactions (including with various AEDs that are commonly used as adjunctive therapy forfocal-onset seizures) [1,16].

Thus, with more and more physicians prescribing this drug for seizure control, the demand for TDM of lacosamide is increasing. Therefore, we modified and validated a simple gradient HPLC method for the quantitative determination of serum lacosamide level taking clues from literature. The developed method is robust, specific, precise, and accurate. This method can be directly adopted in quality control laboratories for routine analysis with respect to determination and quantification of lacosamide. Unlike other published methods, this method does not incorporate the evaporation step as well as multiple mobile phases for the detection of lacosamide in serum [8,9]. On that account, this method will be suitable for a TDM laboratory providing a routine clinical service for both adults and children and, in addition, can be used to study the pharmacokinetics of lacosamide in specific patients and clinical settings.

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AUTHORS CONTRIBUTION

Both the authors have contributed toward the preparation, review, and editing of the manuscript. Ms Jyoti Vishwakarma – data collection and analysis and writing of the manuscript; Dr Renuka Munshi – data analysis, review and editing of the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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REFERENCES

1. Hoy SM. Lacosamide: A review in focal-onset seizures in patients with epilepsy. CNS Drugs 2018;32:473-84. DOI: 10.1007/s40263-018-

0523-7, PMID 29785508
2. Medicine I. Medicine Brands for Generic 'Lacosamide'. Available from: https://www.medicineindia.org/brands-for-generic/3213/lacosamide [Last accessed on 2022 Jun 10].
3. Rogawski MA, Tofighy A, White HS, Matagne A, Wolff C. Current understanding of the mechanism of action of the antiepileptic drug lacosamide. Epilepsy Res 2015;110:189-205. doi: 10.1016/j.eplepsyres.2014.11.021, PMID 25616473
4. Beydoun A, D'Souza J, Hebert D, Doty P. Lacosamide: Pharmacology, mechanisms of action and pooled efficacy and safety data in partial-onset seizures. Expert Rev Neurother 2009;9:33-42. doi: 10.1586/14737175.9.1.33, PMID 19102666
5. Cawello W. Clinical pharmacokinetic and pharmacodynamic profile of lacosamide. Clin Pharmacokinet 2015;54:901-14. doi: 10.1007/s40262-015-0276-0, PMID 25957198
6. Patsalos PN, Spencer EP, Berry DJ. Therapeutic drug monitoring of antiepileptic drugs in epilepsy: A 2018 update. Ther Drug Monit 2018;40:526-48. doi: 10.1097/FTD.0000000000000546, PMID 29957667
7. Kestelyn C, Lastelle M, Higuet N, Dell'Aiera S, Staelens L, Boulanger P, *et al*. A simple HPLC-UV method for the determination of lacosamide in human plasma. Bioanalysis 2011;3:2515-22. doi: 10.4155/bio.11.261, PMID 22122600
8. Jayasimha N, Reddy VK, Sandeep V, Goud ES. Development and validation of RP-HPLC for assay of lacosamide injection. World J Pharm Pharm Sci 2015;4:1067-74.
9. Devi MS, Varma DP, Rani GT, Srinivas N. Liquid chromatographic assay of lacosamide in human plasma using liquidliquid extraction. Int J Pharm Pharm Sci 2014;6:530-3.
10. Chakravarthy VK, Shankar DG. HPLC method for determination of lacosamide S(-)Enantiomer in bulk and pharmaceutical formulation. Rasayan J Chem 2011;4:744-52.
11. Shah S, Vasantharaju SG, Karthik A, Muddukrishna BS. Development and validation of stability-indicating assay method for lacosamide by RP-HPLC. Elixir Int J 2011;38:4174-7.
12. Raul SK, Mahapatra AK, Kumar BV, Patnaik AK. Stability indicating RP-HPLC method for the estimation of lacosamide in bulk and pharmaceutical dosage form. J Chem Pharm Res 2013;5:732-9.
13. European Medicines Agency. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology. Energy Market Authority of Singapore. Vol. Q2. Amsterdam: European Medicines Agency; 2005. p. R1. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf [Last accessed on 2022 Jun 10].
14. Reimers A, Berg JA, Burns ML, Brodtkorb E, Johannessen SI, Landmark CJ. Reference ranges for antiepileptic drugs revisited: A practical approach to establish national guidelines. Drug Des Dev Ther 2018;12:271-80. doi: 10.2147/DDDT.S154388, PMID 29467570
15. Harris JA, Murphy JA. Lacosamide and epilepsy. CNS Neurosci Ther 2011;17:678-82. doi: 10.1111/j.1755-5949.2010.00198.x, PMID 20950330
16. Becerra JL, Ojeda J, Corredera E, Giménez JR. Review of therapeutic options for adjuvant treatment of focal seizures in epilepsy: Focus on lacosamide. CNS Drugs 2011;25 Suppl 1:3-16. doi: 10.2165/1159572-S0-000000000-00000, PMID 22141347