



**Solubility Determination of Lacosamide by HPLC with Application to the
Biopharmaceutics Classification System**

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Abstract

Literature survey reveals that no method has been reported so far for the solubility determination of Lacosamide in the form of API by HPLC. The objectives of this work were to classify Lacosamide in the proposed BCS, and integrate drug biopharmaceutical properties with formulation variables to predict the in vivo performance of Lacosamide IR formulations. Aqueous drug solubility of crystalline Lacosamide was determined at 37°C by using the Water, 0.1 N HCL, pH 4.5 Acetate buffer, pH 3.0 Phosphate buffer, pH 6.8 Phosphate buffer, pH 7.5 Phosphate buffer as media prescribed in USP. Solution of API was prepared by shaking 200mg of API in 250 ml of media for 2 hrs and then 5 ml of it was diluted to 25 ml. A simple, specific and accurate reversed phase HPLC method was developed for the solubility determination of Lacosamide, using an Inertsil C-8, 150 mm x 4.6 mm, 5 µm column at 40°C and a mobile phase composed of Acetonitrile and KH₂PO₄ buffer (15:85) pH 3.0 adjusted with *o*-phosphoric acid. The retention time of Lacosamide was found to be at 7.16 min. This solubility determination provides an in vivo context of formulation and dissolution changes and a basis to identify acceptable formulation ranges.

Keywords: *Lacosamide, BCS, IR formulations, Biopharmaceutical properties, HPLC.*



Introduction

An objective of the BCS approach is to determine the equilibrium solubility of a drug substance under physiological pH conditions. The pH-solubility profile of the test drug substance should be determined at $37 \pm 1^{\circ}\text{C}$ in aqueous media with a pH in the range of 1-7.5. A sufficient number of pH conditions should be evaluated to accurately define the pH-solubility profile. The number of pH conditions for a solubility determination can be based on the ionization characteristics of the test drug substance. The Biopharmaceutics Classification System (BCS) is a scientific framework for classifying drug substances (active ingredient) based on their aqueous solubility and intestinal permeability. When combined with the dissolution of the drug product, the BCS takes into account three major factors that govern the rate and extent of drug absorption from Immediate Release (IR) solid oral dosage forms: dissolution, solubility, and intestinal permeability.

According to the BCS, drug substances are classified as follows:

Class 1: High Solubility - High Permeability

Class 2: Low Solubility - High Permeability

Class 3: High Solubility - Low Permeability

Class 4: Low Solubility - Low Permeability

The solubility class boundary is based on the highest dose strength of an IR product that is the subject of a bio waiver request. A drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1-7.5.



The permeability class boundary is indirectly based on the extent of absorption (fraction of dose absorbed, non-systemic BA) of a drug substance in humans and directly on measurements of the rate of mass transfers across human intestinal membrane.^[1]

Lacosamide(R)-2-acetamido-N-benzyl-3-methoxypropionamide is a functionalized amino acid and its molecular formula is $C_{13}H_{18}N_2O_3$. Lacosamide is a white to light yellow non-hygroscopic powder, sparingly soluble in water and slightly soluble in acetonitrile and ethanol at both 25°C and 37°C. Lacosamide is Antiepileptic drug approved as adjunctive therapy for the treatment of partial-onset seizures in adults that enhances slow inactivation of voltage-gated sodium channels, thereby stabilizing hyperexcitable neuronal membranes and inhibiting repetitive neuronal firing.^[2-3]

According to the FDA biowaiver guidance, a waiver for in vivo BE studies can be requested for solid, orally administered immediate-release drug products (>85% release in 30 min) containing highly soluble drug over the pH range from 1 to 7.5 (dose to solubility ratio < 250 ml) which are also highly permeable (fraction absorbed $\geq 90\%$) (2). Thus, for BCS class I drug substances, an in vivo bioequivalence study could be replaced by generating suitable in vitro dissolution data.^[4-7]

Materials and Methods

Lacosamide of pharmaceutical grade was purchased from Ami Life Science Pvt. Ltd. and was certified to contain 99.78% (w/w) on dried basis. Acetonitrile and Methanol used were of HPLC grade and were purchased from Rankem, (RFCL Limited) New Delhi, India. The HPLC system (Shimadzu Corporation, Japan), model Shimadzu VP, consisted of a system



controller (SCL-10AVP), on-line degasser (DGU-14A), low-pressure gradient flow control valve (FCV-10ALVP), solvent delivery module (LC-10ADVP), auto injector (SIL-10ADVP), column oven (CTO-10AVP), UV – VIS detector (SPD-10AVP) and CLASS – VP software version 6.14 SP1. The chromatographic analysis was performed using an Inertsil C-8, 150 mm x 4.6 mm, 5 mm column, electronic balance (Mettler Toledo UMT2), a pH meter (Labindia Pvt. Ltd), a sonicator (5510, Branson Ultrasonics Corporation), a hot air oven (Jeiotech OV-11).

Preparation of Mobile Phase, Media, Sample and Standard Solutions

Dissolve 1.36 g of potassium dihydrogen phosphate in 1000ml of double distilled water. Adjust the pH to 3.0 ± 0.05 using orthophosphoric acid and filter with 0.45 μ m filter. Prepare a mixture of Acetonitrile and buffer (15:85). This mixture was sonicated for 10 min and used as mobile phase. Water, 0.1 N HCL, pH 4.5 Acetate buffer, pH 3.0 Phosphate buffer, pH 6.8 Phosphate buffer, pH 7.5 Phosphate buffer as media are prepared as prescribed in USP. Different solution of API was prepared by shaking 200mg of API in 250 ml of different media for 2 hrs and then 5 ml of it was diluted to 25 ml. For preparation of stock solution weigh accurately about 200.0 mg of Lacosamide (working / reference standard or API sample) into a 250 ml volumetric flask, add to it 180 ml of media and shaking on shaker to dissolve, make the volume up to the mark with media, mix thoroughly. Further dilute 5 ml of above solution to 25 ml with media to obtain a solution containing 160 ppm of Lacosamide. The HPLC analysis was performed on reversed-phase high-performance liquid chromatographic system with isocratic elution mode using an Inertsil C-8, 150 mm x 4.6 mm, 5 μ m column at



40°C and a mobile phase composed of Acetonitrile and KH_2PO_4 buffer (15:85), pH 3.0 adjusted with *o*-phosphoric acid with injection volume 10 μl , run time 12 min, 1.5 ml/min flow rate at 215 nm using UV detector. Three replicate determinations of solubility in each pH condition were made.

Results and Discussion

Lacosamide was classified as highly soluble; since solubility at each pH exceeded the threshold value of 0.80 mg/ml (maximum dose divided by 250 ml). The results for solubility are summarized in Table 1. The chromatogram of the standard and API sample in different media showed from fig. 2 to fig. 13. In the proposed study, solubility of Lacosamide was determined as per FDA guidelines. The developed method was found to be simple, sensitive and selective for analysis of Lacosamide. The method was successfully used for determination of solubility of Lacosamide. This solubility determination provides an *in vivo* context of formulation and dissolution changes and a basis to identify acceptable formulation ranges.

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Table 1: Solubility Data for Lacosamide API

S. No.	Media	Solubility (mg/ml)	Solubility (mg/100 ml)	Solubility (mg/250 ml)
1.	Water	0.80	80.03	200.07
2.	0.1N HCl	0.80	80.08	200.2
3.	pH 3.0 Phosphate Buffer	0.80	80.05	200.12
4.	pH 4.5 Acetate Buffer	0.80	80.14	200.35
5.	pH 6.8 Phosphate Buffer	0.80	80.19	200.47
6.	pH 7.5 Phosphate Buffer	0.80	80.12	200.3

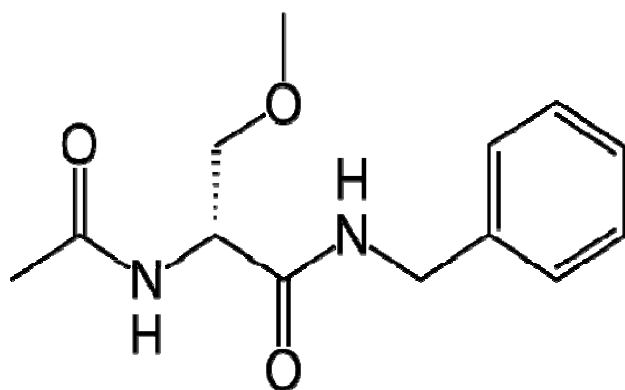


Fig. 1: Structure of Lacosamide

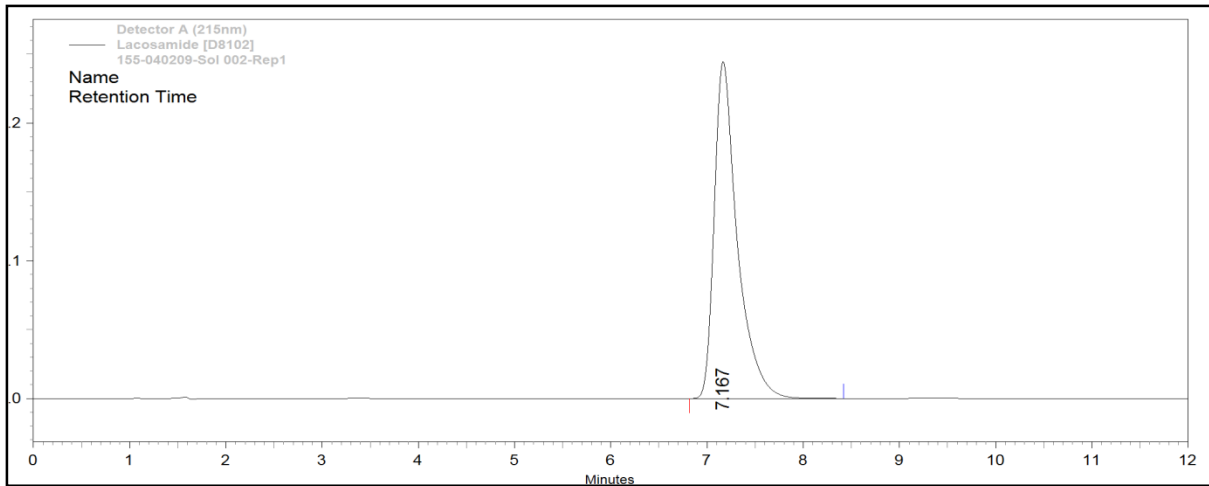


Fig. 2: Chromatogram depicting Standard Solution of Lacosamide in Water ($t_R = 7.16$ min.)

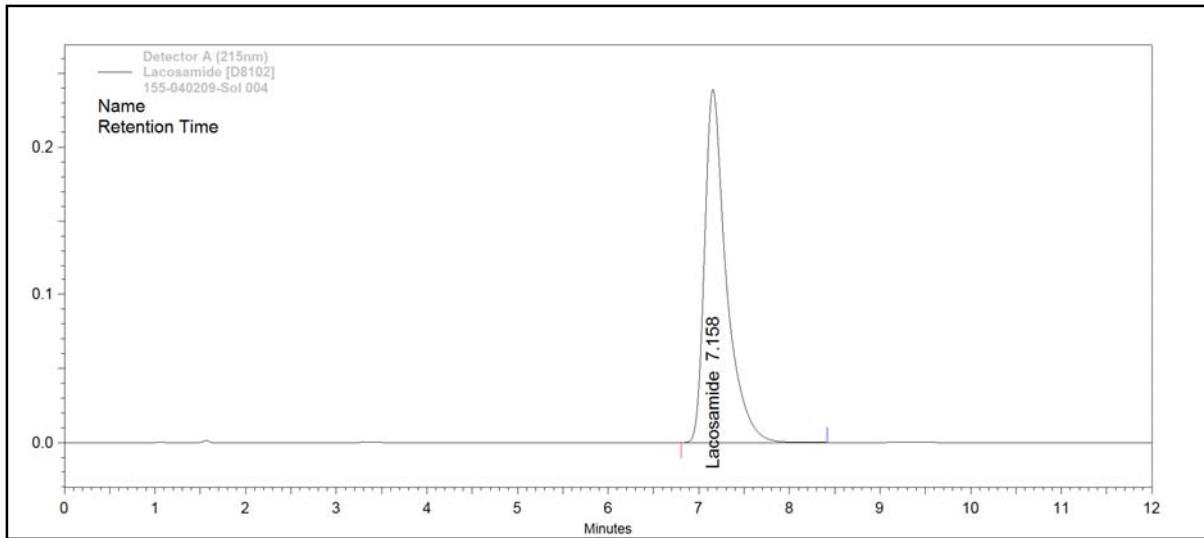


Fig. 3: Chromatogram depicting Sample Solution of Lacosamide in Water ($t_R = 7.15$ min.)

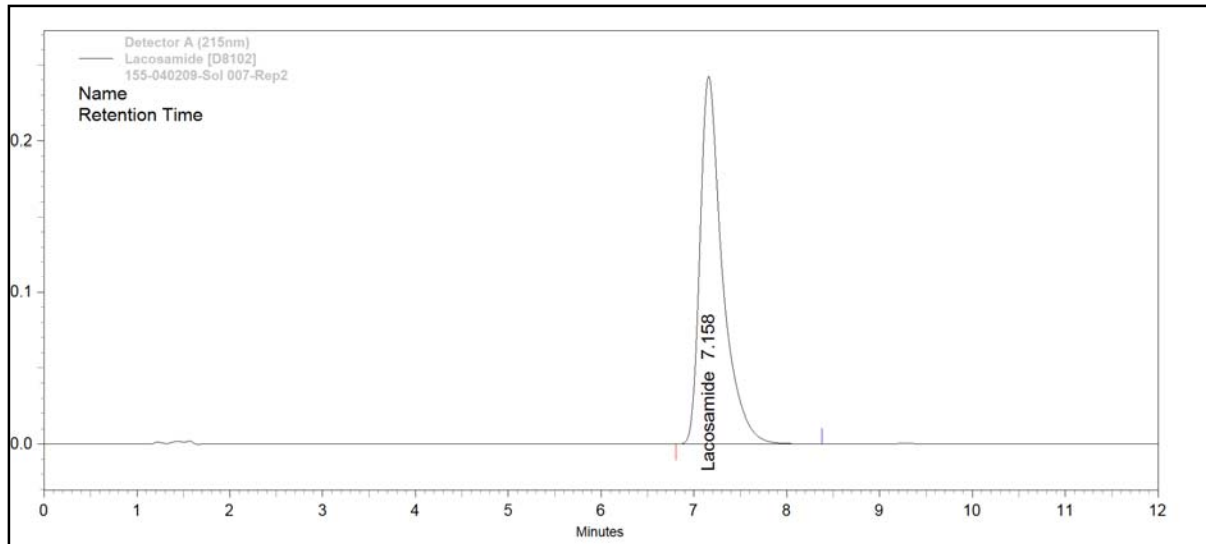


Fig. 4: Chromatogram depicting Standard Solution of Lacosamide in 0.1N HCl ($t_R = 7.15$ min.)

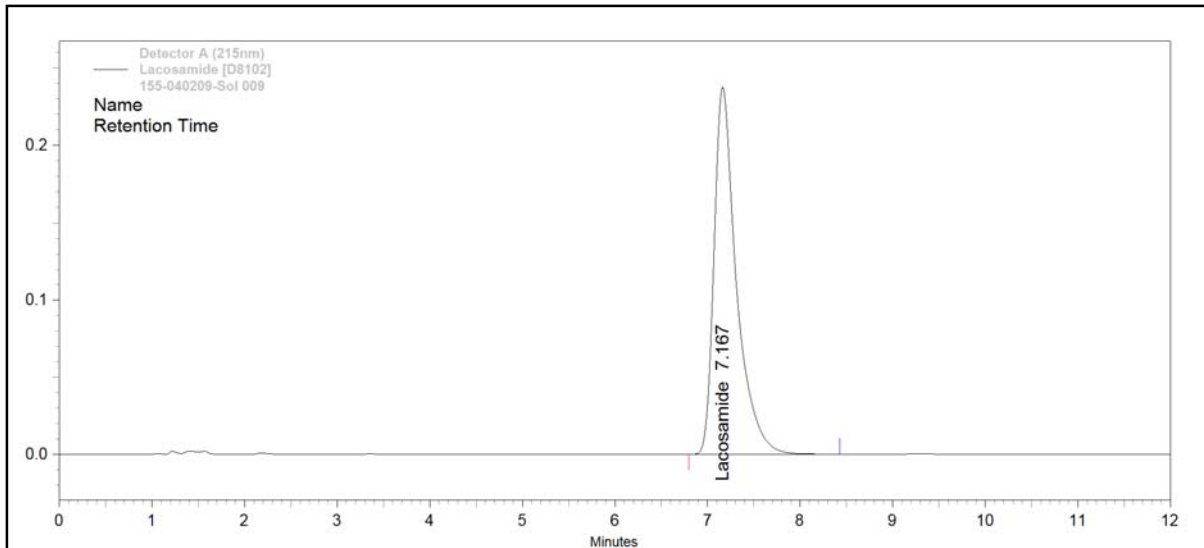


Fig. 5: Chromatogram depicting Sample Solution of Lacosamide in 0.1N HCl ($t_R = 7.16$ min.)

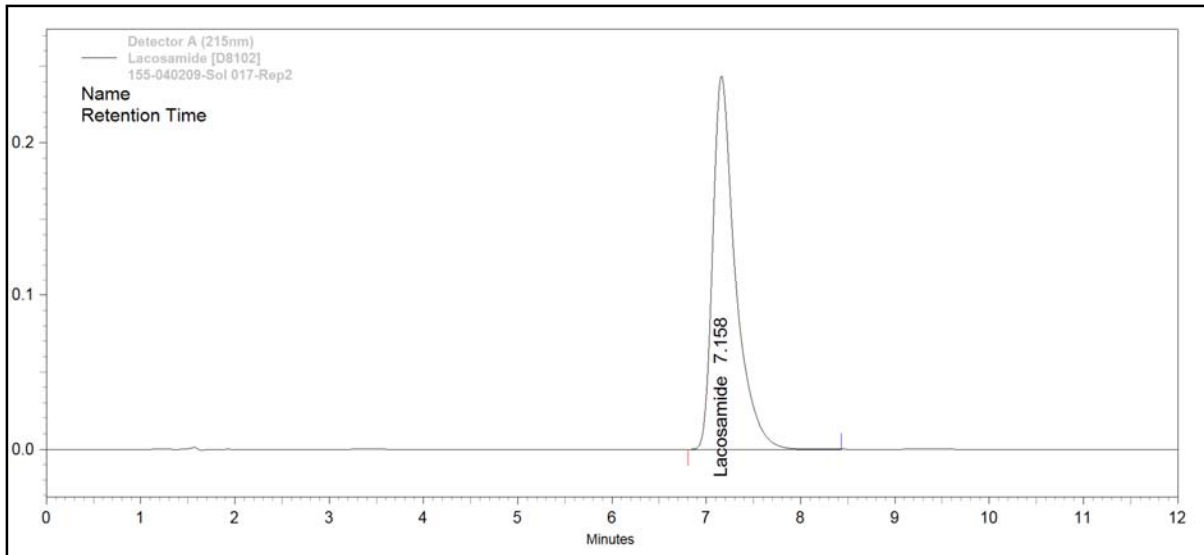


Fig. 6: Chromatogram depicting Standard Solution of Lacosamide in pH 3.0 Phosphate Buffer ($t_R = 7.15$ min.)

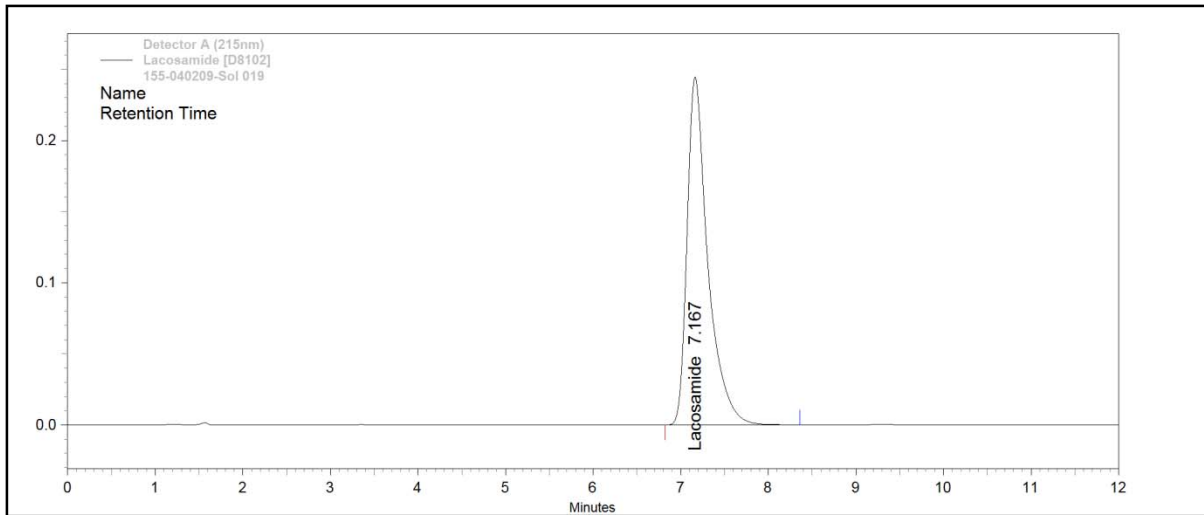


Fig. 7: Chromatogram depicting Sample Solution of Lacosamide in pH 3.0 Phosphate Buffer ($t_R = 7.16$ min.)

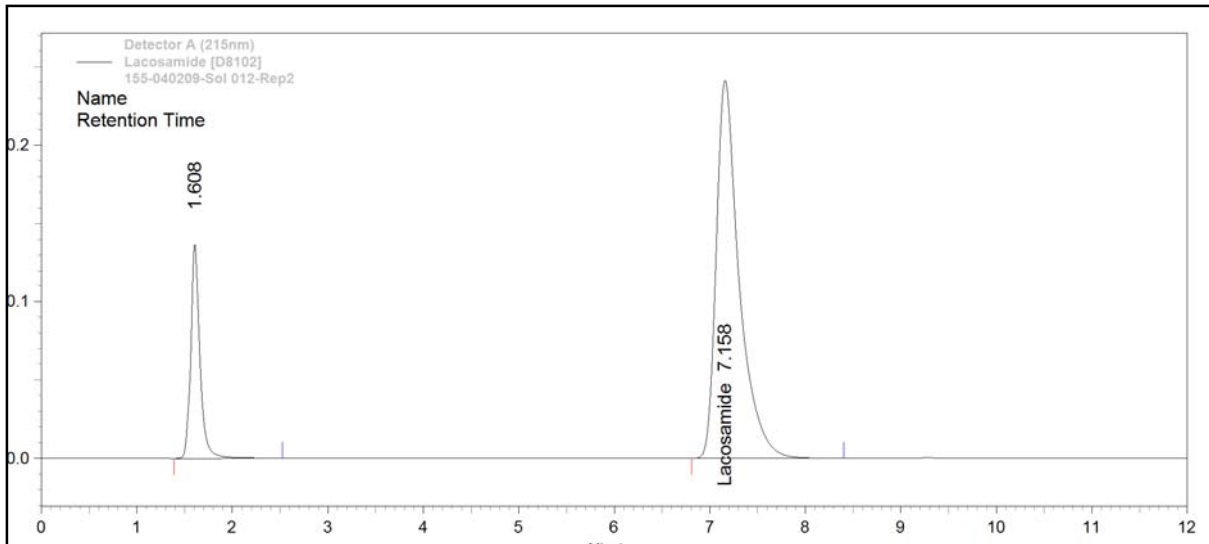


Fig. 8: Chromatogram depicting Standard Solution of Lacosamide in pH 4.5 Acetate Buffer ($t_R = 7.15$ min.)

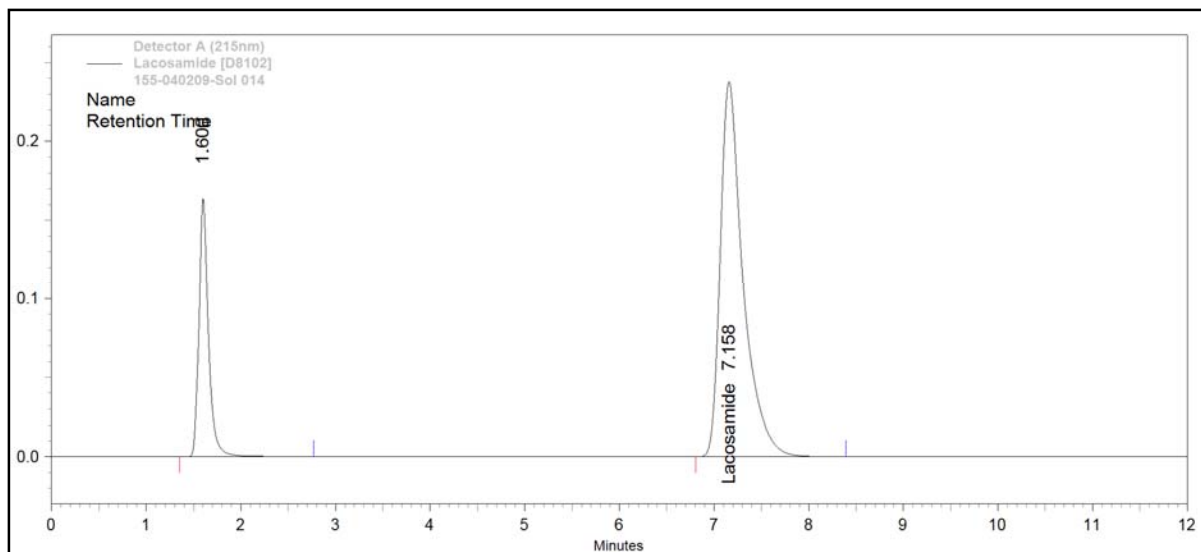


Fig. 9: Chromatogram depicting Sample Solution of Lacosamide in pH 4.5 Acetate Buffer ($t_R = 7.15$ min.)

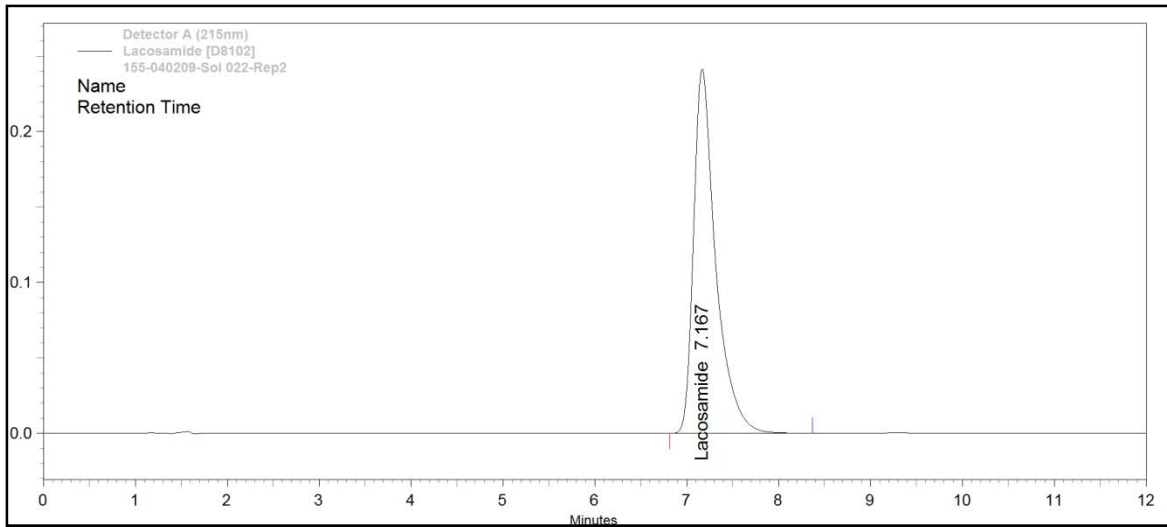


Fig. 10: Chromatogram depicting Standard Solution of Lacosamide in pH 6.8 Phosphate Buffer ($t_R = 7.16$ min.)

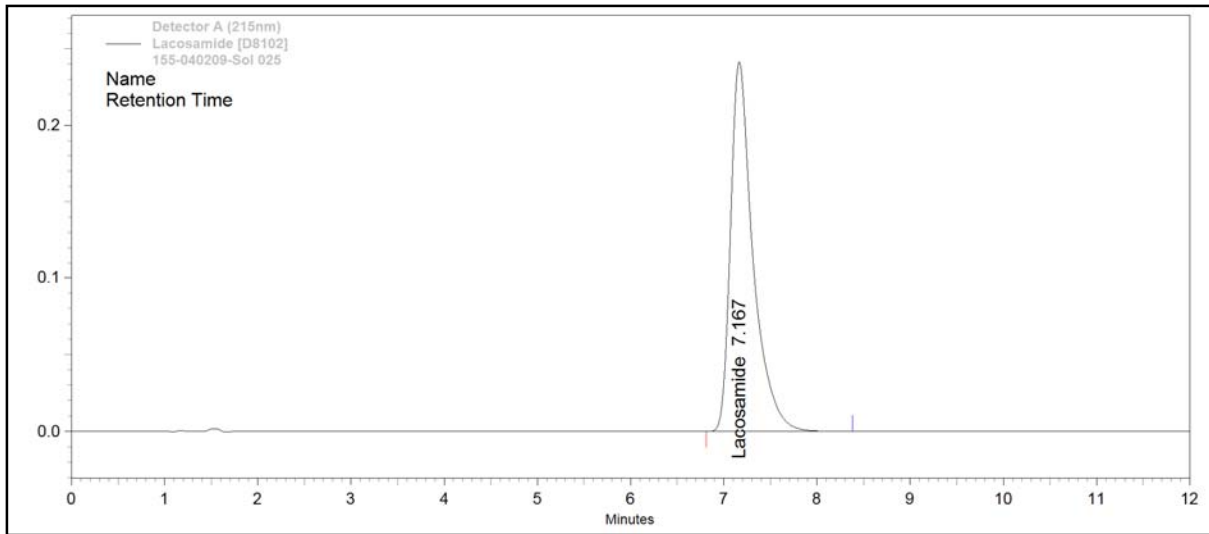


Fig. 11: Chromatogram depicting Sample Solution of Lacosamide in pH 6.8 Phosphate Buffer ($t_R = 7.16$ min.)

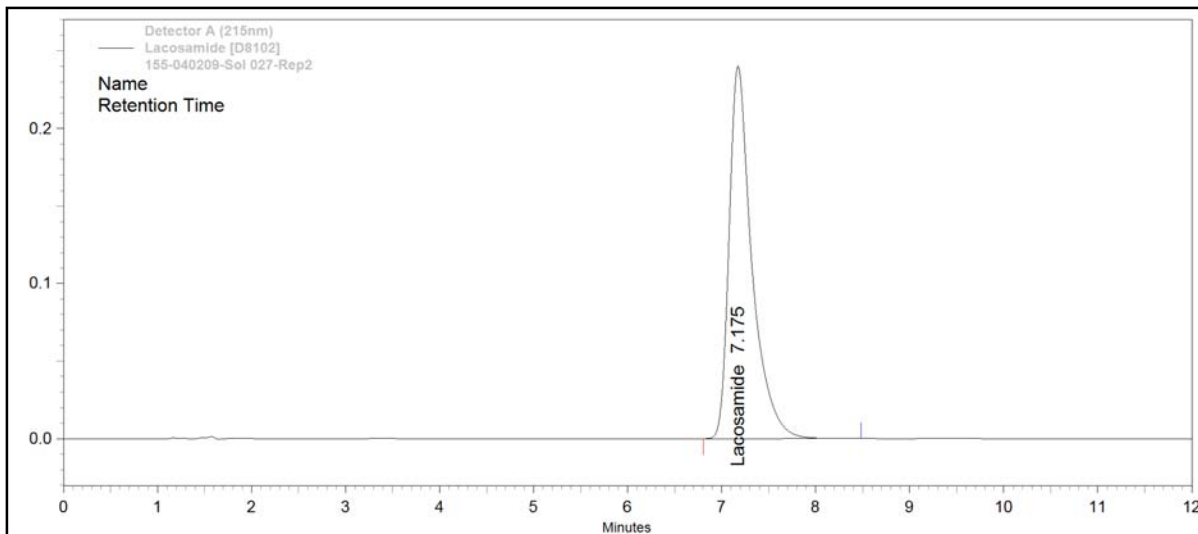


Fig. 12: Chromatogram depicting Standard Solution of Lacosamide in pH 7.5 Phosphate Buffer ($t_R = 7.17$ min.)

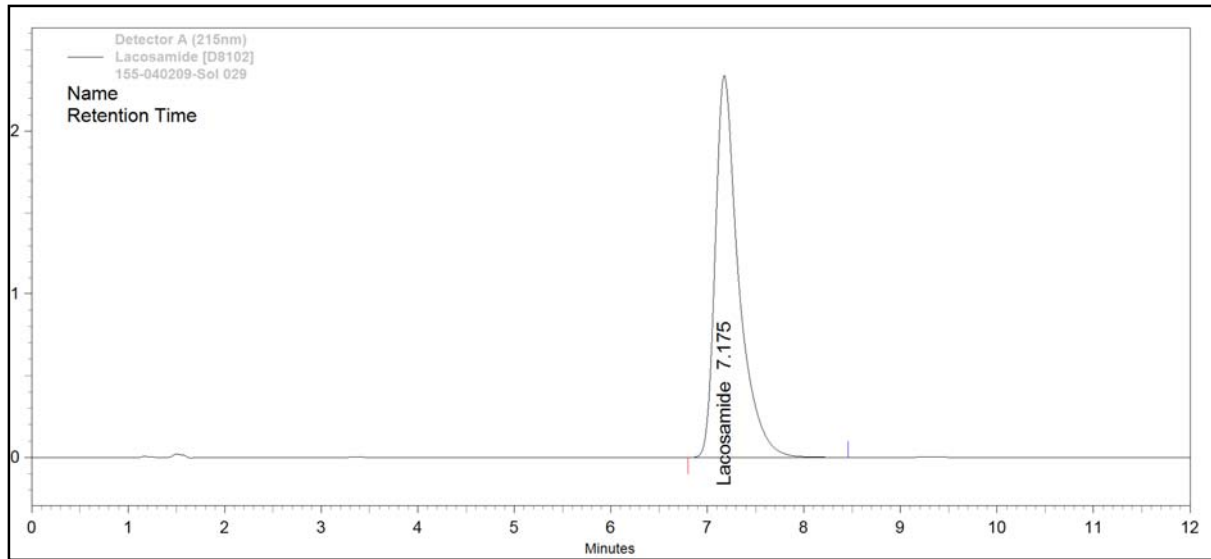


Fig. 13: Chromatogram depicting Sample Solution of Lacosamide in pH 7.5 Phosphate Buffer ($t_R = 7.17$ min.)