METHOD DEVELOPMENT AND QUANTITATIVE ESTIMATION OF ACAMPROSATE CALCIUM USING RP-HPLC

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ABSTRACT

Objective: To develop and validate economic, user friendly, precise, specific and accurate RP-HPLC method for Acamprosate calcium.

Methods: The RP-HPLC method uses Reverse phase C18 column (Comosil) (4.6 X 250mm, 5µm), a mobile phase of acetonitrile, (0.5%) at flow rate 0.7 ml/min, at 25^{o}C ± 2^{o}C and detection wavelength was set at 210 nm.

Results: The developed method of RP-HPLC displayed linearity in the range of 2.9 to 22.6 part per million. The specificity obtained indicated non-interference from other impurities. The LOQ for acamprosate calcium obtained to be 39:1 and LOD for acamprosate calcium obtained to be 13:1.

Conclusion: A sensitive, specific and accurate method with good separation of acamprosate calcium was developed. This economic and rapid quality control method has practical applications in both research and industrial QC laboratories.

KEYWORDS: Acamprosate calcium, RP-HPLC, Validation, Limit of Quantitation, Limit of Detection.

INTRODUCTION:

Acamprosate calcium (calcium 3-acetamidopropane-1- sulfonate) is U.S. FDA approved drug used in treatment of chronic alcohol dependence[1-3]. Chemically, acamprosate calcium (Figure 1) is C10H20CaN2O8S2 with molecular weight of about 400.48 g/mol. It acts as a glutaminergic N-methyl-D-aspartate receptor antagonist and gamma-aminobutyric acid (GABA) type A receptor agonist thereby stabilizing Central Nervous System. It is white, odorless powder with fairly good solubility in methanol and water[4,5].

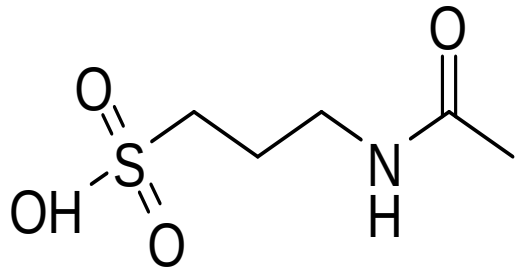

Figure 1. Structure of Acamprosate calcium

The comprehensive literature survey reported capillary zone electrophoresis method[6-8], Liquid Chromatography mass spectrometry (LCMS) method for analysis of Acamprosate calcium bioanalytically, LC-fluorometric, and electrochemical detection in human plasma, dog plasma, and urine[9-15]. RP-HPLC method has also been reported in past for the quantitative estimation of acamprosate calcium[16]. In this research, a new economical, precise, simple and accurate RP-HPLC method on acamprosate calcium in pure form is reported.

MATERIALS AND METHODS:

Chemicals and Reagents

Pure sample of Acamprosate calcium with more than 99% of purity and Impurity-B (Formylacamprosate calcium) was obtained from Ind-Swift Labs, Mohali. Milli Q water (HPLC grade) was obtained from Lab Tech Millipore Ltd, Banglore, Methanol (HPLC grade), Ortho-phosphoric acid (AR grade), acetonitrile (HPLC grade) and Triethylamine (AR grade) were obtained from Merck Chemicals.

Instruments

HPLC analysis was performed on HPLC (Waters 515), Reverse phase C18 column Comosil (4.6 X 250mm, 5µm), UV spectrophotometer Shimadzu Pharmaspec UV-1700. A pH meter (Eutech Instruments), Sonicator (Flexit Jour Labs (P) Ltd.), Analytical micro balance (Sartorius) and Millipore (Labtech) were used in this study.

Methods

Preparation of working standard stock solution

10 mg of accurately weighed Acamprosate calcium and 5ml of methanol was taken in 10ml of volumetric flask. The solution was sonicated and volume was mark upto 100ml with methanol. The resulting solution was of 1000ppm concentration of analyte.

Selection of wavelength

Appropriately diluted analyte solutions of Acamprosate calcium were scanned in UV range from 200-400nm to obtain the spectra and determine the wavelength. The suitable wavelength 210nm was selected for further method.

Chromatographic conditions

The method used for RP-HPLC validation of Acamprosate calcium uses a reverse phase C18 column, Comosil (4.6 X 250mm, 5µm), mobile phase consisting of acetonitrile, (0.5%). The flow rate of mobile phase was set at 0.7 ml/min, at 25oC ± 2oC and the injected volume was 20 µl for every injection with run time of 60minutes. The detection wavelength was set at 210 nm.

Buffer Preparations

The buffer solution (0.5% of TEA) was prepared by adding 5ml of Triethylamine (TEA) in 1000ml of distilled water and adjusted to pH 4 with ortho-phosphoric acid.

Preparation of System suitability solution

10mg of impurity-B / Acamprosate calcium (reference standard/working standard) were weighed and transferred in 10ml volumetric flask. Milli Q water (5ml) was added in the same volumetric flask and sonicated to dissolve the sample and volume was made up to the mark with diluent (Solution-1). Again, 50 mg acetic acid reference standard/working standard was added in a 50 ml volumetric flask and was diluted with 10ml of diluent to and volume was made up to the mark with diluent (Solution-2). Lastly, 1ml of both (solution-1 and solution-2) were taken in a 100ml volumetric flask and made up the volume up to the mark with diluent.

Preparation of Reference solution

100mg of acamprosate calcium reference standard/working standard and 100mg of acetic acid reference standard/working standard were weighed and taken into a 100ml volumetric flask. 20ml of diluent was added and sonicated to dissolve the sample and made up the volume up to the mark with diluent. This solution was further diluted by taking 1.0ml of this solution to 100ml with diluent.

Preparation of Test solution

500mg sample of Acamprosate calcium was weighed and taken into a 50ml volumetric flask. 20.0ml of diluent was added and sonicated just to dissolve the sample and made up the volume up to the mark with diluent.

Procedure of HPLC

The C18 column was flushed with acetonitrile:water (30:70) for 45 minutes at 0.7ml/min of flow rate. Then the column was equilibrated with mobile phase for 60 minutes at similar flow rate. 20µl of system suitability solution was injected and the chromatogram was run at mentioned chromatogram conditions. Similarly, the blank solution, reference solution and test solution was also run at similar chromatogram conditions.

RESULTS AND DISCUSSION:

The chromatogram results for system suitability solution are illustrated in Table 1 and Figure 2. Similarly, the chromatogram results for blank and test solution are illustrated in Figure 3, 4 and the results for test solution are depicted in Table 2.

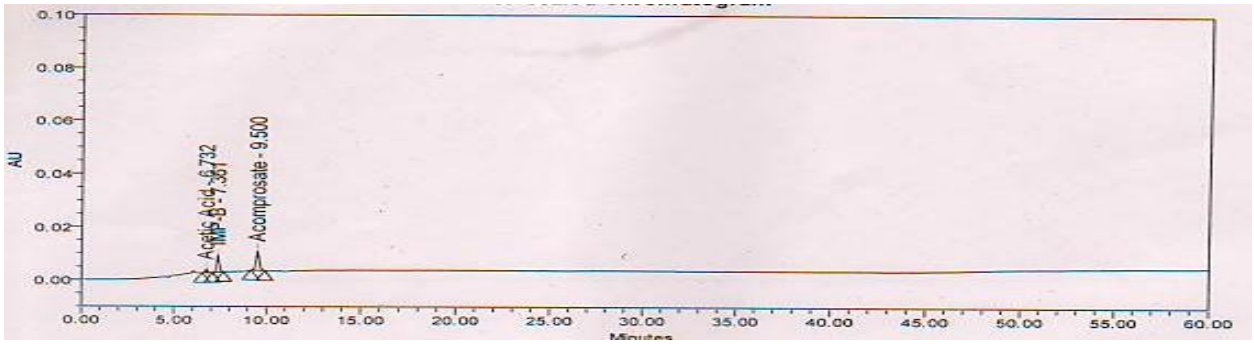

Figure 2. Chromatogram of system suitability solution

Table 1. System suitability studies results

Name of Impurities	**Retention time (min)**	**% Area**	**Symmetry Factor**
Acetic acid	6.732	7.01	1.0
Impurity-B	7.361	40.26	1.1
Acamprosate Ca	9.500	52.71	1.1

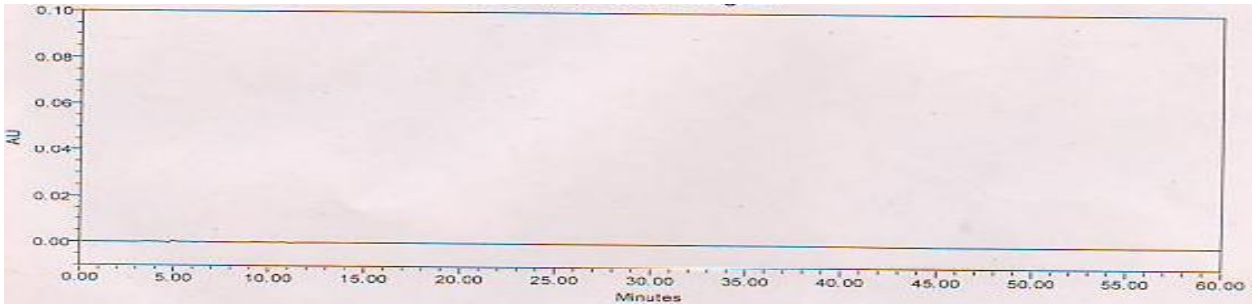

Figure 3. Chromatogram of Blank solution

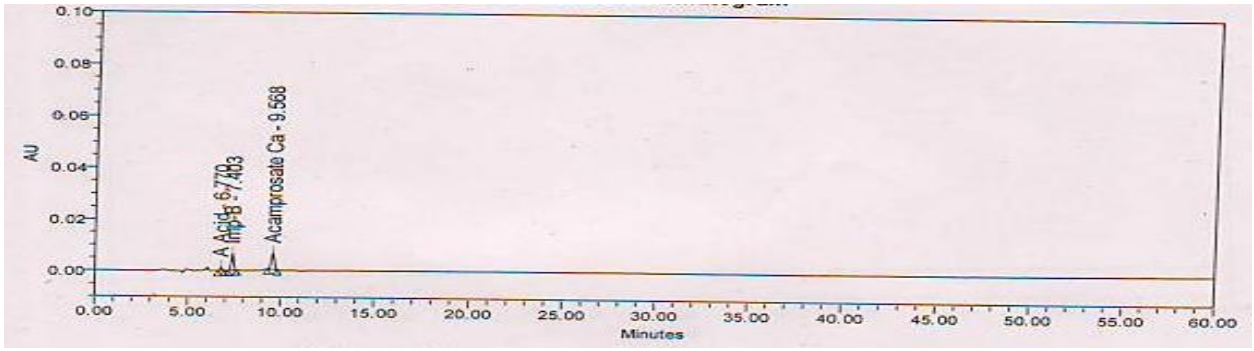

Figure 4. Chromatogram of Test solution

Table 2. Test solution Results

S. No.	Peak name	Retention time(min)	Area	%Area
1.	Acetic acid	6.770	9995	6.86
2.	Impurity-B	7.403	6282	43.13
3.	AcamprosateCa	9.568	7285	50.01

Method Validation

Validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. The developed chromatographic method for assay of Acamprosate calcium was found to adequate so it was subjected to validation that includes Linearity, Limit of detection, Limit of quantitation, Specificity and Robustness.

Specificity

Specificity is performed to check that whether our method is specific or not. And from the above chromatograms, it is clear that there is no peak in the blank and the peaks are only obtained in the test solution. Hence, the RP-HPLC method is concluded to be specific.

Linearity

The linearity was performed at LOQ level. The linearity solution was injected into the HPLC system. The mean area at each level was calculated and graph of mean area versus concentration was plotted (Table 3 and Figure 5). The correlation co-efficient (r2) was calculated and recorded to be 0.9998 which met the acceptance criteria (between 0.990 and 1.0) and hence the method is said to be linear.

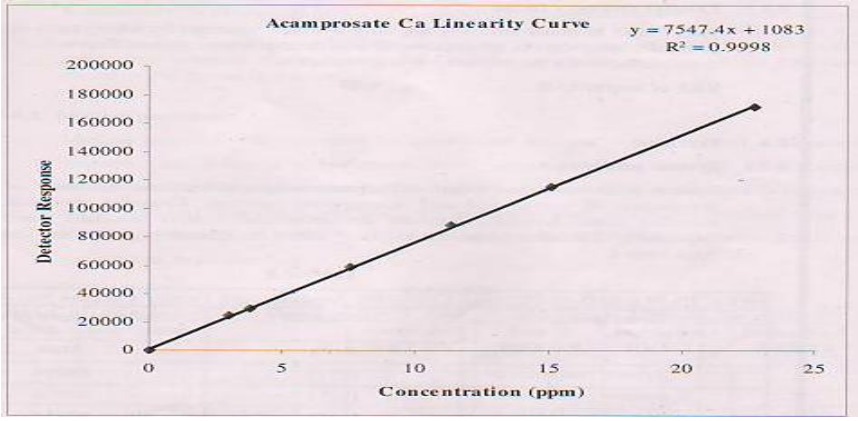

Figure 5.Linearity graph of Acamprosate calcium

Table 3. Linearity study of Acamprosate calcium

Conc. (%)	Conc. (in ppm)	Peak Area
0	0	0
20	2.9	24141
25	3.7	29454
50	7.5	58666
75	11.3	87820
100	15.1	115218
150	22.6	171661

Limit of detection (LOD)

Limit of detection (LOD) is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value). For this, main drug substance (Acamprosate Calcium) and all the impurities was injected at a very low concentration level (0.2 ppm) into the HPLC system and then the LOD was calculated by using the formula **S/N** (Signal to Noise ratio) where S is the height of the peak due to drug substance in the chromatogram and N is the noise obtained from the blank. For an ideal method, **S/N** ratio for LOD should be more than 3.

First, blank solution was injected into the HPLC system and noise was calculated from the blank chromatogram (Figure 6).

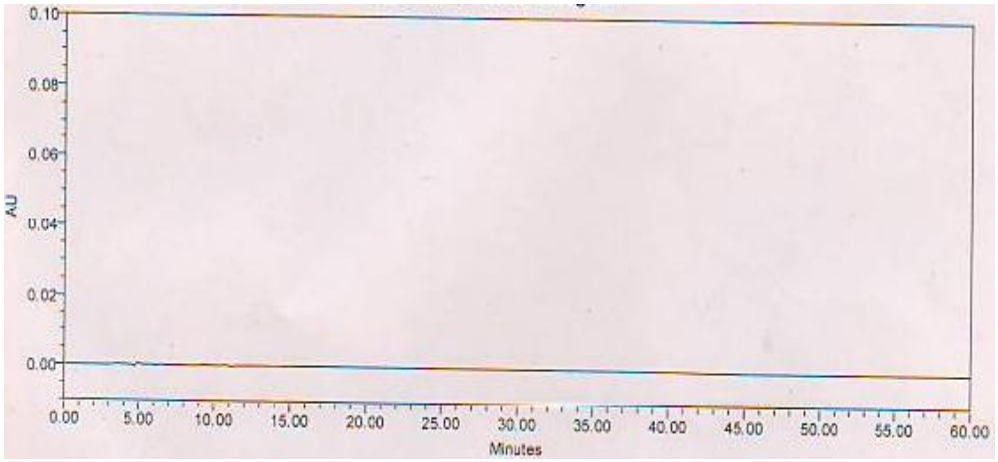

Figure 6.Chromatogram of Blank

Then, Acamprosate Calcium and all the impurities were injected at 0.2 ppm (LOD level) and the following chromatogram was obtained (Figure 7).

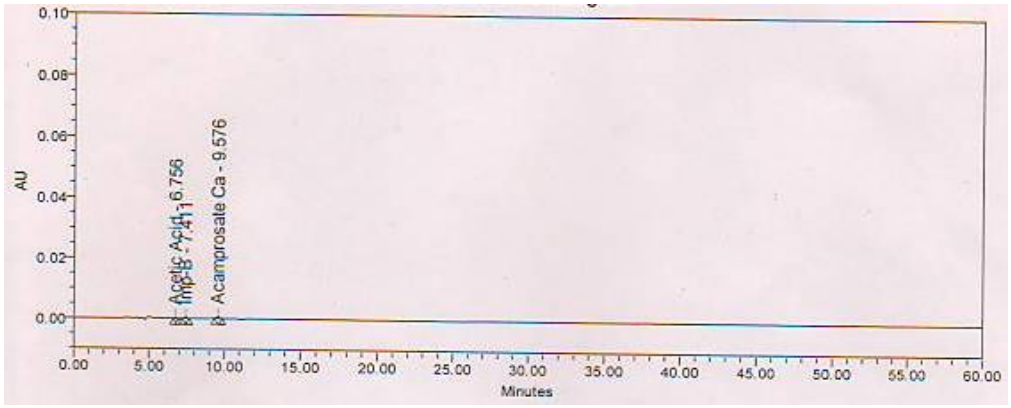

Figure 7.Chromatogram of LOD (0.2ppm)

From the chromatogram of blank, noise is obtained and from the chromatogram of LOD, height of each peak is obtained, which is considered as signal, so from that data, S/N for each peak is calculated (Table 4).

Table 4. Limit of detection results

S. No.	**Peak name**	**Retention time (min)**	**Height (µV)**	**S/N ratio**
1	Acetic acid	6.756	258	5

2	Impurity-B	7.411	567	12
3	AcamprosateCa	9.576	612	13

Limit of Quantitation (LOQ)

The limit of quantitation can be defined as the smallest concentration of analyte which gives a response that can be accurately quantified. For this, main drug substance (Acamprosate Calcium) and all impurities were injected at a very low concentration level (0.6 ppm) into the HPLC system and then the LOQ was calculated by using the formula **S/N** (Signal to Noise ratio). For an ideal method, **S/N** ratio for LOD should be more than 10. In case of LOQ, the limit is almost 3 times more than LOD. So, the LOQ solution at 0.6ppm (3 times more than LOD value) was injected.First, blank solution was injected into the HPLC system and the noise was calculated from the blank solution (Figure 8).

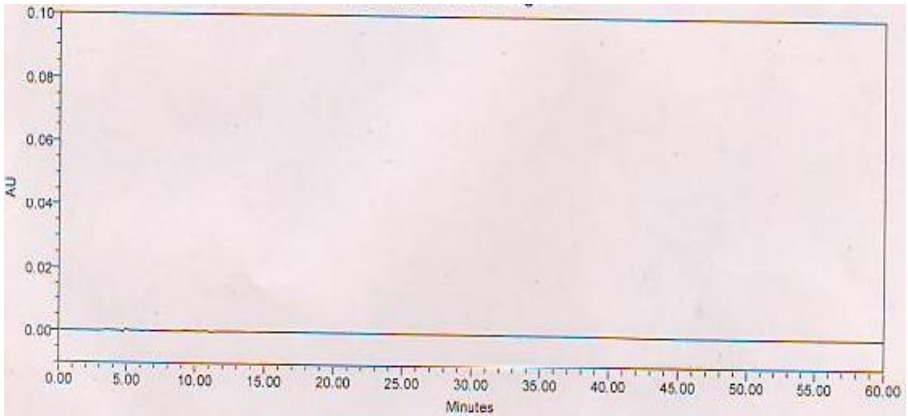

Figure 8.Chromatogram of Blank

Then, Acamprosate Calcium and all the impurities were injected at 0.6 ppm (LOQ level) and the chromatogram obtained (Figure 9).

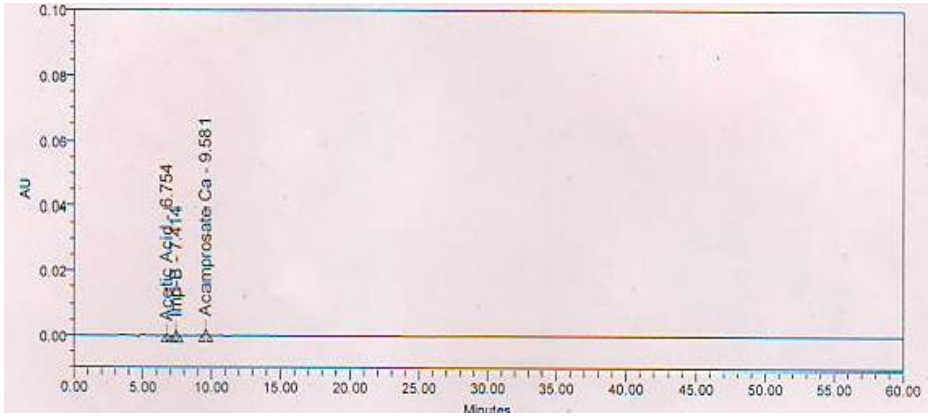

Figure 9.Chromatogram of LOQ (0.6ppm)

From the chromatogram of blank, noise is obtained and from the chromatogram of LOQ, height of each peak is obtained, which is considered as signal. So from that data, S/N for each peak is calculated as depicted in table 5.

Table 5. LOQ results

S. No.	Peak name	Retention time (min)	Height(µ V)	S/N ratio
1	Acetic acid	6.754	533	11
2	Impurity-B	7.414	1757	37
3	Acamprosate calcium	9.581	1901	39

Robustness

The robustness is the measure of the capacity of an analytical method to remain unaffected by small but deliberate variations in method parameters and provides as indications of its reliability during normal usage. The sample was analyzed separately by deliberate changes in the analytical method.

a. pH

The pH of the buffer was changed from 3.9 and 4.1. The chromatograms were recorded as displayed in Figure 10 and 11. The % area is illustrated in Table 6.

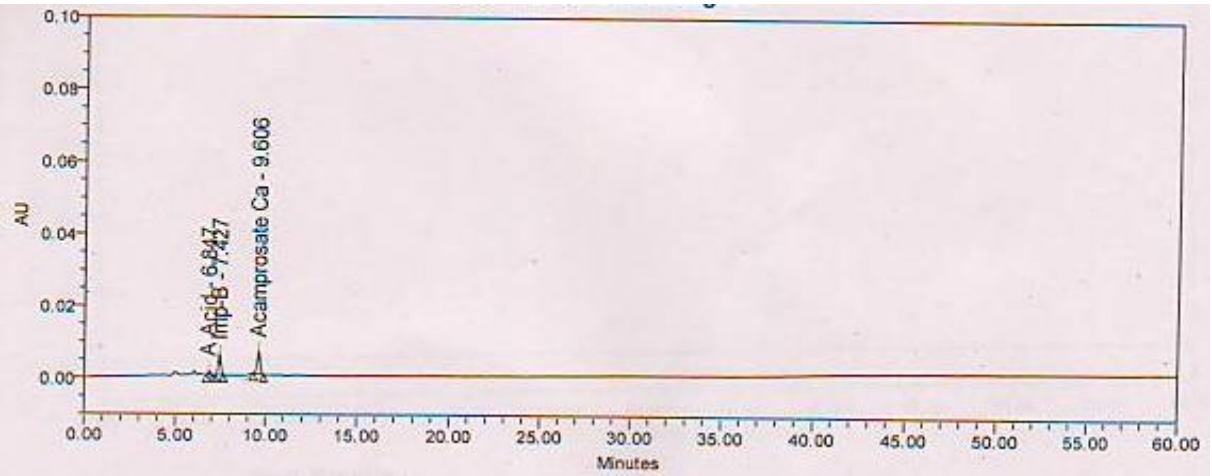

Figure 10.Chromatogram of pH 3.9

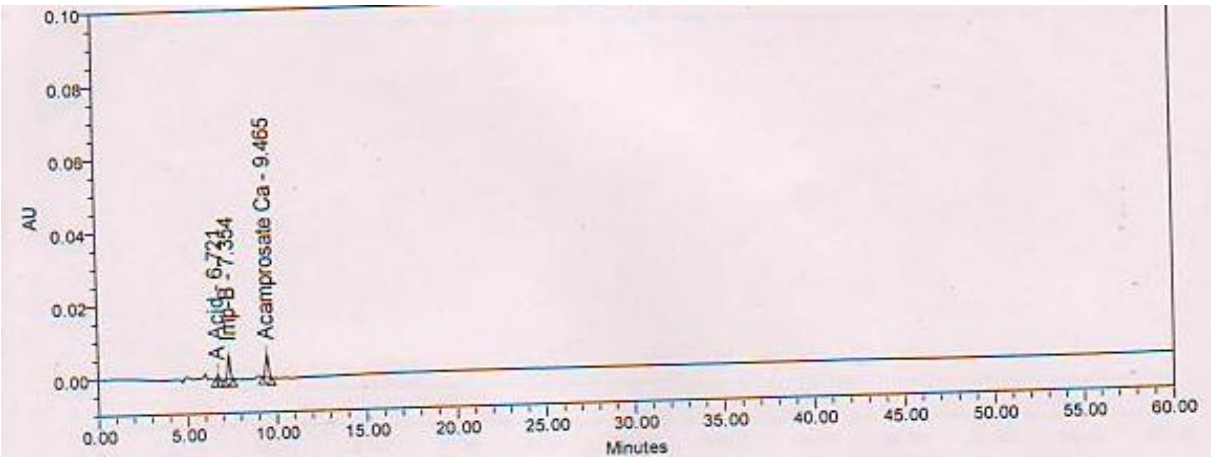

Figure 11.Chromatogram of pH 4.1

Table 6. Area for pH 3.9 and 4.1

Area for pH 3.9			
S. No	**Peak Name**	**Retention Time (min)**	**% Area**
1	Acetic acid	6.847	6.34
2	Impurity B	7.427	40.39
3	Acamprosate calcium	9.606	53.27
Area for pH 4.1			
S. No	**Peak Name**	**Retention Time (min)**	**% Area**
1	Acetic acid	6.721	6.63
2	Impurity B	7.354	43.21
3	Acamprosate calcium	9.465	50.16

b. Temperature

The column temperature was decreased and increased. The chromatograms were recorded as displayed in Figure 12 and 13. The % area is illustrated in Table 7.

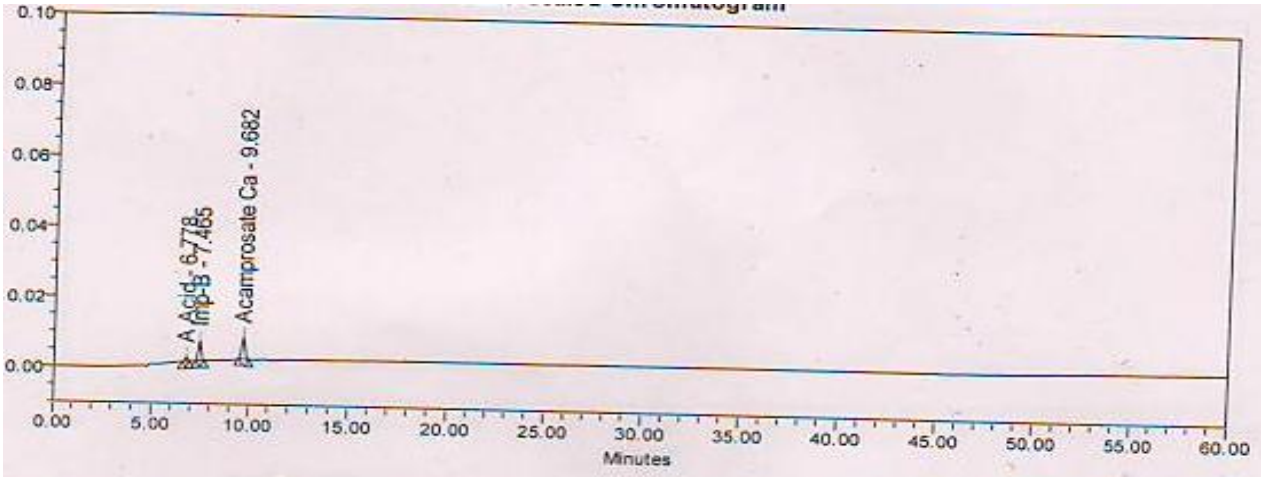

Figure 12.Chromatogram of decrease temperature

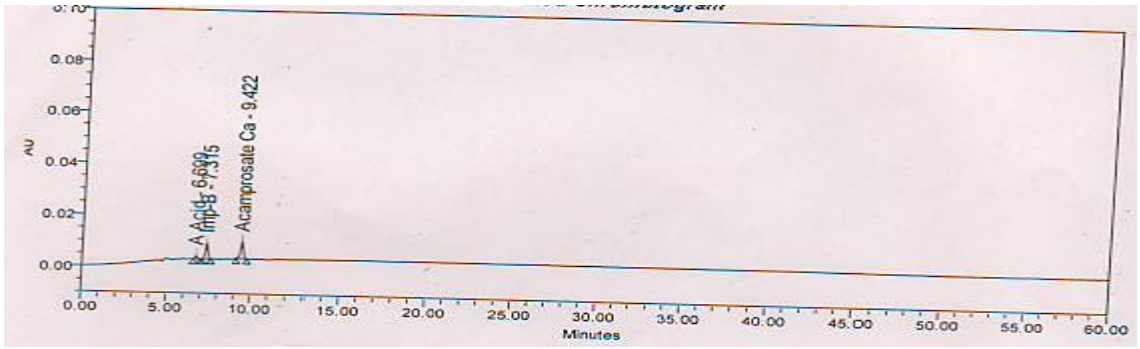

Figure 13.Chromatogram of increased temperature

Table 7.Area for decrease and increase in temperature

Area for Decreased Temperature			
S. No	**Peak Name**	**Retention Time (min)**	**% Area**
1	Acetic acid	6.778	7.13
2	Impurity B	7.455	40.46
3	Acamprosate calcium	9.682	52.4
Area for Increased Temperature			
S. No	**Peak Name**	**Retention Time (min)**	**% Area**
1	Acetic acid	6.699	6.96
2	Impurity B	7.315	39.96
3	Acamprosate calcium	9.422	53.08

CONCLUSION:

A Reverse Phase High Pressure Liquid Chromatography method has been developed and validated as per ICH guidelines for acamprosate calcium and its impurities. The validation process parameters were within the limits. The symmetry factor value was found to be 1.0. Theoretical plate count was 7559. The LOQ and LOD were also in the acceptable limits i.e., LOQ was 39:1 and LOD was 13:1. The linearity was found to be 0.999. The resolution between acetic acid and impurity B was 2.3 and 2.6. Hence, it was concluded that reported method is sensitive, specific, nd accurate method with good separation of acamprosate calcium. This economic and rapid quality control method has practical applications in both research and industrial QC laboratories.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

REFERENCES:

1. Rezaeia M, Ali Ramazania, FaridehGouranloub. Synthesis and Purification of Acamprosate Calcium and its Evaluation by RP-HPLC in Pharmaceutical Dosage Forms. Iranian Journal of Pharmaceutical Sciences. 2020; 16(1): 19-28.
2. Aseervadamma M, Babu CA, Ramanjaneyulu K, Kumar JS, Basha M. Method Development & Validation of Acamprosate Tablet Dosage Form by RP-HPLC. World Journal of Pharmacy and Pharmaceutical Sciences. 2014; 3(12): 984-994.
3. Kirankumar A, Mamatha B, Sasikala M, Monika S, Ranganayakulu D. Validated UV spectrophotometric method development and stability studies of acamprosate calcium in bulk and tablet dosage form. International Journal of Pharmtechnology Research. 2013; 5(3): 1241-1246.
4. Babua C, SreenivasaRao B, Suresh Reddy KVN, Naganjaneyulu B. Development and Validation of HPLC Assay Method for the AcamprosateCa in Commercial Tablets. Indian Journal of Advances in Chemical Science. 2013; 2(1): 46-49.
5. Saivin S, Hulot T, Chabac S, Potgieter A, Durbin P, Houin G. Clinical pharmacokinetics of acamprosate. Clinical Pharmacokinetics. 1998; 35(5): 331-345.
6. Blanchin MD, Baalbak B, Bosc N, Fabre H. Short-end injection technique in capillary electrophoresis for dissolution testing of tablets. AnalyticaChimicaActa. 2000; 415: 67-73.

7. Fabre H, Perrin C, Bosc N. Determination of homotaurine as impurity in calcium acamprosate by capillary zone electrophoresis. Journal of Chromatography A. 1999; 853: 421-430.
8. Fabre H, Blanchin M, Bosc N. Capillary electrophoresis for the determination of bromide, chloride and sulfate as impurities in calcium acamprosate. AnalyticaChimicaActa. 1999; 381(1): 29-37.
9. Chabenat C, Ladure P, Blanc-Continsouza D, Boismare F, Boucly P. Determination of calcium acetylhomotaurinate by liquid chromatography with fluorimetric and electrochemical detection. Journal of Chromatography B: Biomedical Sciences and Application. 1987; 414: 417- 422.
10. Chabenat C, Ladure P, Moore N, Boucly P, Boismare F. Application of an analytical method to calcium acetylhomotaurinate determination in urine. Arzneimittelforschung. 1989; 39: 1413-1414.
11. Ghosh C, Jha V, Shinde CP, Chakraborty BS. A LC- MS analysis of acamprosate from human plasma: pharmacokinetic application. Drug Testing and Analysis. 2011; 3(10): 735-742.
12. Girault J, Gobin P, Fourtillan J. Determination of calcium acetylhomotaurinate in human plasma and urine by combined gas chromatography-negative-ion chemical ionization mass spectrometry. Journal of Chromatography B: Biomedical Sciences and Application. 1990; 530: 295- 305.
13. Jayaram-Lindström N, Lindefeldt A, Andersson M, Brundin L, Reid MS, Franck J. Acamprosate determinations in plasma and cerebrospinal fluid after multiple dosing measured by liquid chromatographymassspectroscopy:a pharmacokinetic study in healthy volunteers. Therapeutic Drug Monitoring. 2010; 32(4): 489-496.
14. Kanchana MK, Babu RC, Nagiat TH, Mukkanti K, Prakash K, Challa BR. Quantification of Acamprosate in human plasma by LC-ESIMS/ MS with solid phase extraction:Application to a bioequivalence study. Journal of Pharmaceutical Research. 2013; 7: 389-396.
15. Rhee YS, Park JH, Park S, Park CW, Ha JM, Jeong KW, Lee DS, Park ES. Analysis of Acamprosate in Beagle Dog Plasma by LCMS-MS. Archives of Pharmacal Research. 2008; 31: 1035-1039.

16. Bharghavi L, Maheshwari M, Kartheek N.; Kumar A. A. RP-HPLC Method Development And Validation For The Quantitative Estimation Of Acamprosate Calcium In Tablets. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(6): 582-585.