



Synthesis and Purification of Acamprosate Calcium and its Evaluation by RP-HPLC in Pharmaceutical Dosage Forms

Mehdi Rezaei^a, Ali Ramazani^{a,*}, Farideh Gouranlou^b

^aDepartment of Chemistry, University of Zanjan, Zanjan, Iran, ^bDepartment of Bioscience and Biotechnology, Malek Ashtar University of Technology, Tehran, Iran

Abstract

The purpose of this study is Acamprosate Calcium synthesis of 3-Aminopropane-1-sulfonic acid (Homotaurine) and validation of assay test in the delayed-release tablets by HPLC without organic solvents. The assay method by HPLC was found to be linear in the concentration range of 50 to 200 $\mu\text{g/mL}$. Successful separation was achieved by isocratic elution on a Phenomenex[®] C18 column (250 mm \times 4.6 mm, 5 μm). The mobile phase was triethylammonium phosphate buffer pH: 4.0 (100%) at the flow rate of 1 mL/min using UV detection at 210 nm, column oven temperature 25°C and injection volume 20 μL . The analytical results were validated by recovery studies. The percentage recovery method was found to be 99.36-100.60%. The LOD and LOQ were found to 0.012 $\mu\text{g/mL}$ and 0.042 $\mu\text{g/mL}$. All the parameters of validation were in the acceptance range. This developed method was successfully applied for estimate the amount of Acamprosate Calcium in the tablets.

Keywords: Synthesis, Evaluation, Acamprosate Calcium, RP-HPLC, Method development, Pharmaceutical.

Corresponding Authors: Ali Ramazani, Department of Chemistry, University of Zanjan, Zanjan, Iran
Tel: (+98) 24-33052572
Email: aliramazani@znu.ac.ir
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1. Introduction

Acamprosate Calcium (Figure 1) with chemically name Calcium bis [3-(acetylamino) propane-1-sulphonate] is used in the treatment of Alcohol addicted patients. Molecular

formula is a $\text{C}_{10}\text{H}_{20}\text{N}_2\text{O}_8\text{S}_2\text{Ca}$ and molecular weight is a 400.48 [1].

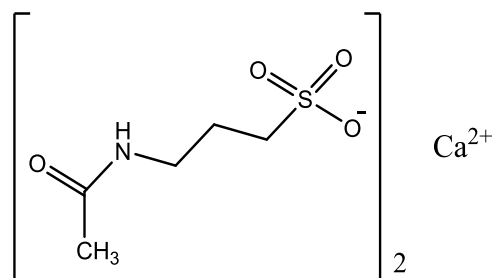


Figure 1. Chemical structure of Acamprosate Calcium.

Acamprosate Calcium is a GABA (γ -aminobutyric acid) agonist which controls alcohol withdrawal symptoms and possesses neuromuscular and vasculometabolic properties. While its specific mechanism of action is not entirely understood, neurotransmission systems involving GABA and its excitatory counterpart glutamate are observed to be thrown out of equilibrium when chronically exposed to alcohol. Acamprosate Calcium, by mimicking GABA's actions and interacting with these systems directly, is thought to help restore this equilibrium [2, 3]. Acamprosate Calcium is a white, odorless or nearly odorless powder. It is freely soluble in water, and practically insoluble in absolute ethanol and dichloromethane [4].

A detailed literature survey reveals that only a few methods are reported previously to determine Acamprosate by isocratic RP-HPLC [5-8]. There are UV spectrophotometric method [9], capillary zone electrophoresis methods [10-12], bioanalytical methods for the analysis of Acamprosate Calcium using LC/MS, LC-ESI-MS/MS, LC-fluorometric and electrochemical detection in human plasma, dog plasma and urine [13-17]. In the present study we report a synthesis, purification and new, cheap, simple, accurate isocratic RP-HPLC method for the quantitative estimation of Acamprosate calcium in the Akamprex[®] delayed-release tablets.

2. Materials and Methods

2.1. Reagents and Chemicals

All Reagents and chemicals were used without further purification and purchased

from commercial sources as follows: 3-Aminopropane-1-sulfonic acid (Sigma), calcium hydroxide (Merck), Acetic acid and Anhydride acetic (Merck), Methanol and Ethanol absolute (Carlo erba), Triethylamine and orthophosphoric acid analysis grade (Merck), LC-grade water has been used by the Puris instrument.

The standard of Acamprosate Calcium (purity, 99.9%) was obtained from Ind-Swift Laboratories Limited, INDIA. Tested samples with brand name Akamprex[®] belonged to the Tekaje Pharmaceutical Company.

2.2. Synthesis of Acamprosate

3-Aminopropane-1-sulfonic acid (1.0 g, 7.19 mmol) and calcium hydroxide (276 mg, 3.72 mmol) are charged into a round bottom flask. Acetic acid (1.0 mL, 17.48 mmol) and purified water (3 mL) were added to reaction mass. The mixture is stirred for 10 minutes at 30°C to obtain a clear solution. Acetic anhydride (2.0 mL, 21.16 mmol) is added dropwise to the reaction mass at 30°C in 10 minutes. The reaction mass is stirred for 2 hours at 30°C. After completion of the reaction and concentrated under vacuum to remove the solvent. The obtained solid is stirred in a methanol (10 mL) for 10 minutes. The solid product is obtained by filtration and washed with ethanol (20 mL) and dried under suction. The material is further dried under vacuum at 50°C for 3 hours to obtain the title compound as white solid.

2.3. Instrumental and Analytical Conditions

The reversed phase-High Performance Liquid Chromatography (RP-HPLC) method was developed on an HPLC system. The HPLC analyses were carried out on Waters e2695 separation module (Waters Corporation, USA) equipped with an autosampler. The separation carried out on Phenomenex® C18 column (250 mm × 4.6 mm, 5µm). Data were analysed by using Empower 3 software. The analyte was monitored with Waters 2998 photodiode array (PDA) detector at 210 nm. The HPLC was operated in an isocratic elution mode. An ultrasonic was used for the sonication of the mobile phase, standard solution, and sample solution.

2.4. Preparation of Solutions

Preparation of solution section is divided into the preparation of mobile phase, standard solution, and sample solution. The details of each section were followed.

2.4.1. Mobile Phase Preparation

The mobile phase solution was prepared by adding 5 mL of triethylamine to 1000 ml of HPLC grade water and later pH was adjusted to 4.0 ± 0.05 by diluted ortho phosphoric acid. The mobile phase was then filtered through 0.45 µm nylon membrane filter and degas it.

2.4.2. Preparation of Standard Solution

The solution was accurately weighed and about 13.2 mg of Acamprosate Calcium (equivalent to 12.0 mg of Acamprosate) reference standards were transferred into a 100 mL volumetric flask, dissolved and diluted with purified water, followed by sonication for

one minutes at room temperature and dilution to volume with purified water. Following this, the solution was filtrated through 0.45 µm cellulose acetate filter.

2.4.3. Sample Preparation

Five Akamprex® delayed-release tablets were weighed and transferred to 500 ml volumetric flask (each tablet contains Acamprosate Calcium 333 mg, equivalent to 300 mg of Acamprosate), dissolved in 350 mL of purified water, sonicated for 30 minutes and diluted up to the mark with the same. The solution was filtered through a filter paper. 2 mL of this solution was pipetted out into a 50 mL volumetric flask and made up to the mark with purified water. The solution was then filtrated through 0.45 µm cellulose acetate filter.

2.5. System Suitability Study

The chromatographic parameters, such as peak area, retention time, theoretical plates and tailing factor were calculated. The peak symmetries were <1.5 and these values are according to the United States Pharmacopeia.

2.6. Method Development

The separation carried out on Phenomenex® C18 column (250 mm × 4.6 mm, 5µm). Data were analysed by using Empower 3 software. The analyte was monitored with Waters 2998 photodiode array (PDA) detector at 210 nm. the mobile phase consisting of triethylammonium phosphate buffer pH: 4.0 (100%). This analysis was done at the 25°C and the flow rate of elution was 1.0 mL/min.

3. Results and Discussion

3.1. Synthesis of Acamprostate

White to off-white powder, Yield: 85%;
HPLC purity: 99.2%; m.p 270°C; ¹H NMR

(D₂O): δ 3.15 (t, 2H), δ 2.80 (t, 2H), δ 1.86 (s, 1H), δ 1.80 (m, 2H); ¹³C NMR (D₂O): 174.13, 48.44, 38.01, 23.93, 21.83 (Figure 2-4).

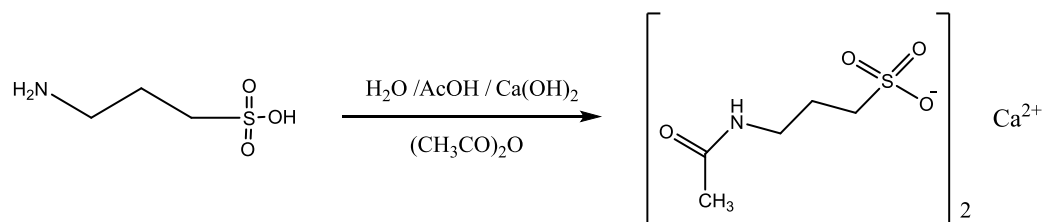


Figure 2. Acamprostate calcium synthesis of homotaurine.

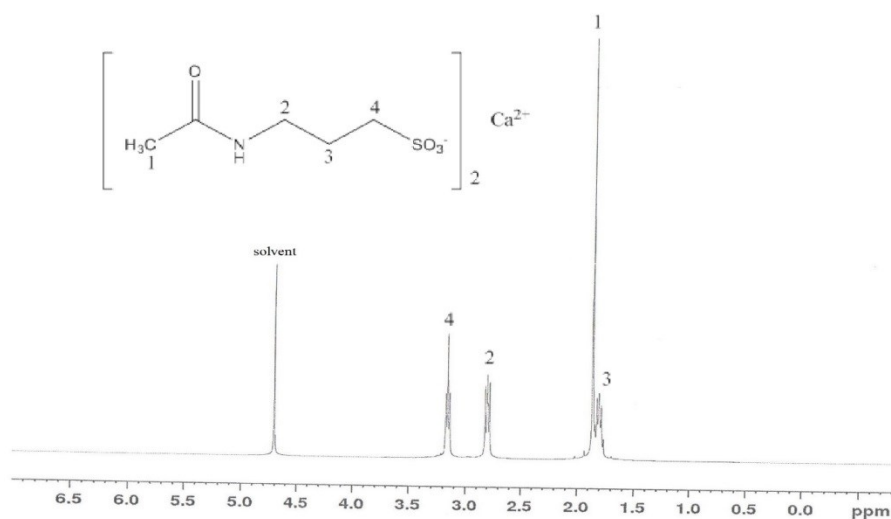


Figure 3. ¹H NMR (400 MHz) spectrum of Acamprostate calcium in D₂O.

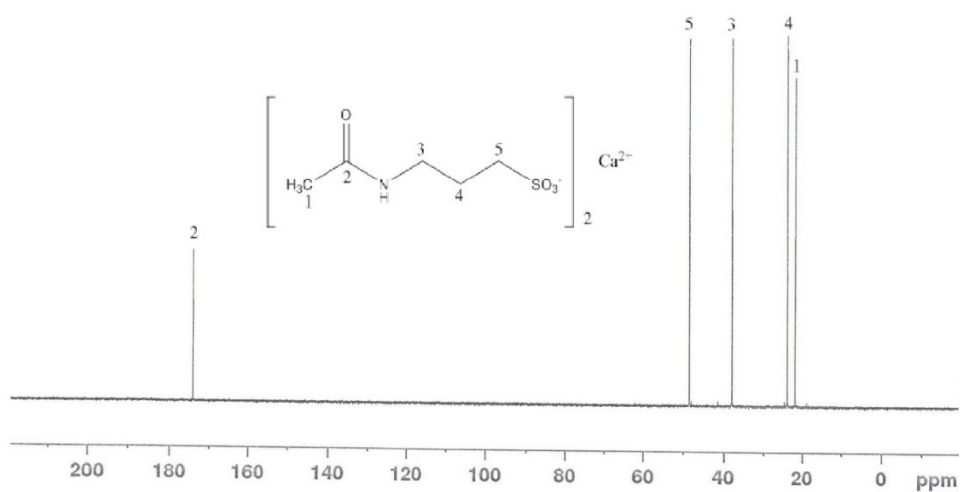


Figure 4. ¹³C NMR (100 MHz) spectrum of Acamprostate calcium in D₂O.

3.2. System Suitability Study

The results of system suitability study were presented in Table 1 and Figure 5. According

to the obtained results the system showed good suitability.

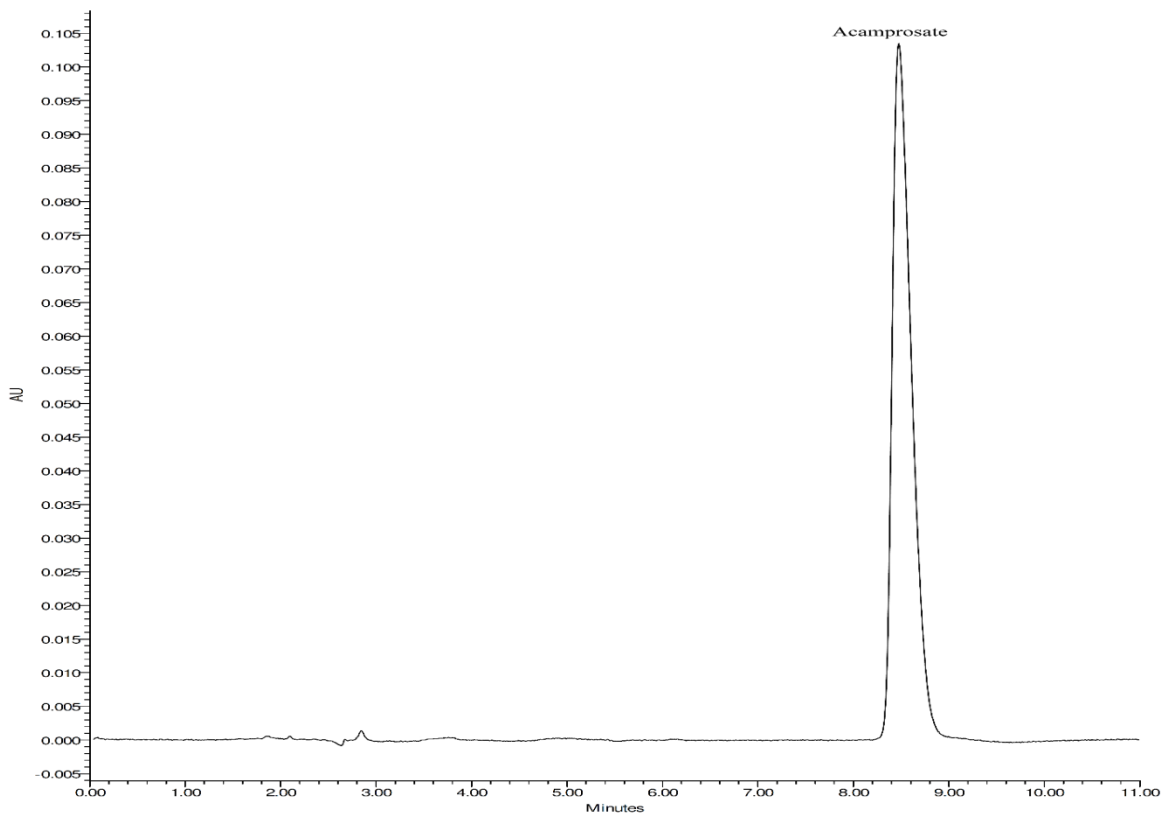


Figure 5. System suitability chromatogram.

Table 1. System suitability parameters of standard chromatogram obtained to Acamprosate

Standard	Retention time	Area	Theoretical plates	USP Tailing
1	8.518	897060.27	9077	1.45
2	8.521	896345.78	9092	1.45
3	8.521	894833.73	9123	1.46
4	8.518	897775.12	9113	1.47
5	8.519	897034.76	9156	1.46
Average	8.519	896609.93	9112.20	1.46
SD	0.0015	1114.18	30.34	0.0084
RSD %	0.018	0.124	0.333	0.574

3.3. Method Validation

All of the analytical validation parameters for the proposed method were determined according to the International Conference on Harmonization (ICH) guidelines. Validation of method was divided into linearity and range, precision, recovery, selectivity, robustness, limit of detection (LOD) and limit of quantification (LOQ) studies. The details of each section were followed.

3.3.1. Linearity and Range

Linearity was checked by preparing standard solutions at six different concentration levels of Acamprosate. A linear response was obtained in the concentrations 50, 75, 100, 125, 150 and 200 $\mu\text{g/mL}$. The linear regression line was used to determine the linearity and concentration of the samples. The calibration curve was developed by plotting concentration of Acamprosate on X-axis and their respective area under the curve (AUC) on Y-axis. The calibration curve is shown in Figure 6.

3.3.2. Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations 100, 125 and 150 $\mu\text{g/mL}$ for Acamprosate three times on the same day. The intermediate precision of the method was checked by repeating studies on three different days (Table 2).

3.3.3. Recovery Studies

Recovery of analytical procedure refers to the closeness of a measured value to a standard or known value. The recovery was analyzed at the three different levels (80%, 100%, and 120 %) in triplicate by the explained method. The recovery studies were carried out by adding a known amount of pure drug Acamprosate Calcium at three different levels to placebo. The combination of placebo is included: Microcrystalline cellulose, Hydroxypropyl methylcellulose, Colloidal silicon dioxide and Magnesium stearate. From the amount of Acamprosate found, percentage recovery was estimated. The results obtained are given in table 3.

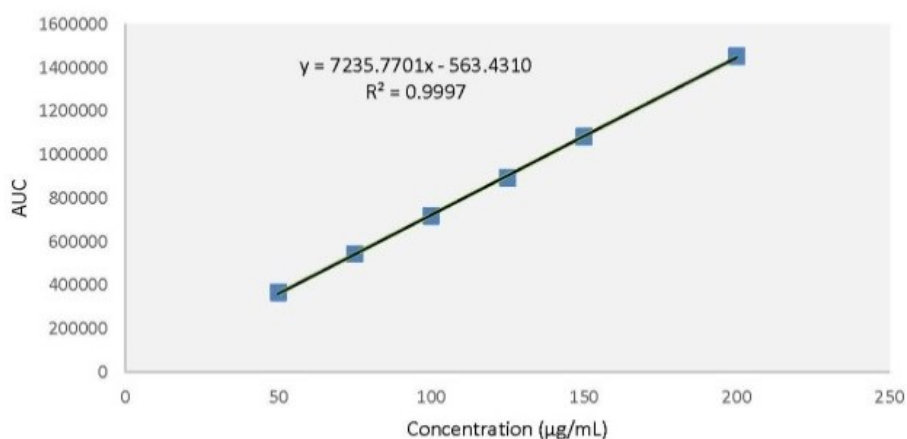


Figure 6. Calibration curve for Acamprosate.

Table 2. Intermediate precision on three different days

Concentration ($\mu\text{g/mL}$)	100	125	150
Day 1			
Mean peak area	738071.77	937776.07	1135927.62
SD	1207.72	1286.98	1729.36
RSD %	0.16	0.14	0.15
Day 2			
Mean peak area	740882.13	939121.11	1135485.14
SD	1074.13	1870.76	466.52
RSD %	0.14	0.20	0.04
Day 3			
Mean peak area	742404.80	940275.68	1139433.37
SD	2197.20	1471.00	1793.36
RSD %	0.30	0.16	0.16

Table 3. Determination of percentage recovery method (n=3)

Level of addition (%)	Amount of pure drug added (mg)	Amount of pure drug Recovered (mg)	Recovery%	Mean recovery%	SD	RSD%
80	240	240.6	100.25			
100	300	301.8	100.60	100.07	0.639	0.639
120	360	357.7	99.36			

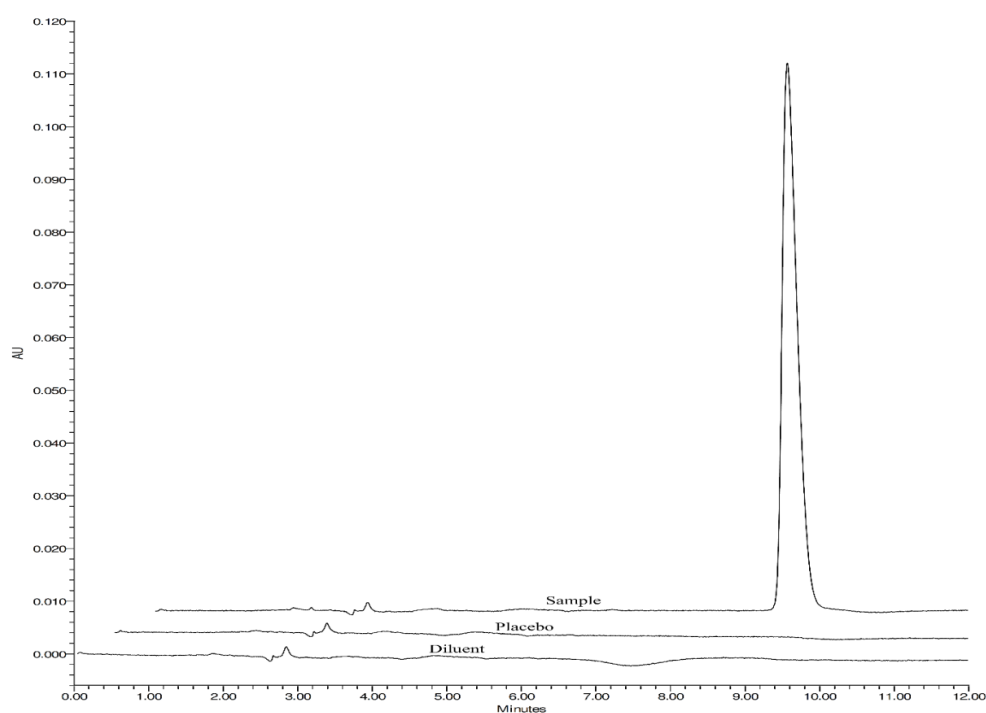


Figure 7. Diluent, placebo and sample solution chromatogram.

3.3.4. Selectivity

Its ability to accurately and specifically measure the analyte of interest without interferences from the blank and other ingredients in the matrix was defined. Figure 7 shows the noninterference excipients with Acamprosate peak.

3.3.5. Detection and Quantitation Limits

The detection limit of an individual analytical procedure is the lowest amount of substance in a sample that can be distinguished but not necessarily quantified as an exact value. The quantitation limit of an individual analytical the procedure is the lowest amount of substance in a sample that can be quantitatively determined with acceptable precision and accuracy. Signal-to-noise ratios were of 3:1 and 10:1 was obtained for the LOD and LOQ. The LOD and LOQ were found to be 0.012 µg/mL and 0.042 µg/mL.

3.3.6. Robustness

In the study, robustness was considered as a measure of the method's capacity to remain unaffected by small, but deliberate changes in chromatographic conditions such as pH, flow

rate and temperature. No significant effect was observed on system suitability parameters such as retention time, tailing factor and theoretical plates. Results of robustness studies are shown in table 4.

4. Conclusion

The aim of this work was to carry out Synthesis, Purification and Novel Evaluation of assay test in the delayed-release tablets by RP-HPLC without organic solvents. The proposed HPLC method is rapid, sensitive, precise, simple and accurate for the determination of Acamprosate Calcium. It can be reliably adopted for routine quality control analysis in the bulk and pharmaceutical dosage forms. All of the analytical validation parameters for the proposed method were determined according to the International Conference on Harmonization (ICH) guidelines.

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Table 4. Robustness testing (n=5).

Parameters	Area	Retention time	Theoretical plates	Tailing factor
A: Flow rate (mL/min)				
0.8	1178407.60	10.529	9961	1.50
1.2	758546.16	7.208	8452	1.43
B: Temperature (°C)				
20	942376.61	9.395	8204	1.50
30	953533.04	7.805	10265	1.45
C: Mobile phase pH				
3.8	904554.69	8.332	9047	1.44
4.2	898903.70	8.294	8998	1.47

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