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Development and Validation of HPLC Assay Method for the Acamprosate Ca in Commercial Tablets

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ABSTRACT

A simple, selective and accurate high performance liquid chromatography method was developed and validated for the analysis of Acamprosate Ca in its Tablet dosage forms. Chromatographic separation was achieved isocratically on ACE phenyl column utilizing a mobile phase of methanol and phosphate buffer (pH adjusted to 7.0 ± 0.05) with 10:90 (v/v). The flow rate is 0.8 mL min^{-1} and analytes are monitered with PDA detector at 210 nm. The detector response for acamprosate was linear over the concentration range from 33µg mL^{-1} to 528 µg mL^{-1} (r^2 = 0.995). The RSD of intra-day and inter-day precision were 0.42% and 0.59% respectively. The accuracy was between 98.17% and 101.77%. There was no interference of the blank on determination of active pharmaceutical ingredients. The LOD and LOQ values for Acamprosate were 0.33 µg mL^{-1} and 1.0 µg mL^{-1} respectively. Acamprosate was subjected to acid, alkali, oxidation and thermal degradations. The proposed method was precise, accurate, specific and sensitive, it can be applied to the quantitative determination of drug in tablets.

Keywords: *Liquid chromatography, Tablet assay, Validation, Acamprosate Ca*

1. INTRODUCTION

Acamprosate Ca is thought to stabilize the chemical balance in the brain that would otherwise be disrupted by alcoholism, possibly by blocking glutaminergic *N*-methyl-D-aspartate receptors, while gamma-aminobutyric acid (GABA) type A receptors are activated [1]. It is an antidipsotropic agent that was approved by the US Food and Drug Administration (FDA) in 2004 for use in alcoholic individuals to decrease alcohol hankering after alcohol detoxification [2]. A monograph on Acamporsate Calcium is being introduced by Lipha involve a potentiometric titration for the assay of the drug and an HPLC method for the determination of Humotaurine, which is both precursor of the synthesis and a potential degradation product of acamprosate [3]. Acamprosate has been commercially available there since 1989, in a 333 mg tablet strength [4]. Literature survey showed that capillary zone electrophorosis methods have been developed for the dissolution and related compounds of Acamprosate tablets [5-6]. Several authors are reported bioanalytical methods for the analysis of Acamprosate as a single drug in human plasma, dog plasma and urine. [7-12]. A literature survey showed that no HPLC method have been previously reported for Acamprosate Ca in its pharmaceutical preparations. In the present study we developed HPLC method for the determination of Acamprosate Ca in tablets. This method was successfully applied to acamprosate 333 mg tablet available in local pharmacies.

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2. EXPERIMENTAL

Corpuscle Research Solutions, Visakhapatnam, India kindly donated Acamprosate Ca standard. The formulation acamprol with 333 mg was purchased from local pharmacies. HPLC grade methanol, analytical grade potassium dihydrogen phosphate, disodium hydrogen orthophosphate anhydrous, citric acid monohydrate, sodium hydroxide, hydrochloric acid and orthophosphoric acid were procured from Merck (Mumbai, India). High purity water was prepared by using milli- Q purification system of millipore.

2.1. Apparatus and Chromatographic Conditions

The chromatographic apparatus consisted of Waters allience system 2695 and detector model 2996, column was a Ace Phenyl, 250 mm × 4.6 mm reversed phase column with 5µ particle size. The mobile phase consisted of mixed phosphate buffer (2.3 g of disodium hydrogen orthophosphate anhydrous and 1.75 g of potassium dihydrogen phosphate in 1000 mL water and pH was adjusted to 7.00 $\pm$ 0.05 by ortho phosphoric acid or 0.1N sodium hydroxide) and methanol in the ratio of 90:10 (*v/v*). Diluent was made of buffer (21.014 g of citric acid monohydrate is dissolved in 150 mL

of 2N sodium hydroxide solution and made up to the 1000 mL and pH is adjusted to 6.8±0.05 by addition of concentrated hydrochloric acid or 2 N sodium hydroxide solution) and methanol in the ratio of 90:10. (*v/v*).

2.2. LC Method Development and Optimization

Before the selection of chromatographic conditions solubility of the Acamprosate was checked with different pH buffers. Finally the citrate buffer (pH = 6.8) was selected as diluent. Mixed phosphate buffer (pH = 7.0) was selected as aqueous part of the mobile phase for the better compatibility with Citrate buffer. The chromatographic conditions were chosen after the test of different proportions of organic solvent. Methanol and acetonitrile are commonly used organic solvents in reversed phase chromatography. But combination of methanol with buffer peak was eluted near dead volume so acetonitrile was selected as organic phase of the mobile phase. Column temperature is maintained at 25 ^{o}C.

2.3. System Suitability

The system suitability test of the chromatographic system was performed before each experimental run to verify the performance of the system with current method and theoretical plates (N=3000), tailing factor (T=1.5) and injection repeatability (RSD=0.9%, n=6) were checked. The resolution between the diluent peak and acamprosate is more than 3.5.

2.4. Preparation of Standard Solution

Standard solution of the drug was prepared from pure compound by dissolving 33mg of Acamprosate Ca working standard in 100 mL volumetric flask with diluent. Working standard solutions were prepared daily. The concentration of working standard was 330 µg mL^{-1}.

2.5. Sample Preparation

The tablets were grinded to provide a homogeneous powder and a quantity equivalent to one tablet was weighed and transferred in to 500 mL dried volumetric flask, containing about 50 mL of methanol and sonicated for 5 minutes with intermittent shaking then 300 mL of diluent and sonicated for 5 minutes with intermittent shaking. Made up to the volume with diluent and mixed well. Further 5 mL of the solution is diluted to 100 mL with diluent and filtered 0.45µm Nylon filter.

3. RESULTS AND DISCUSSION

3.1 Specificity

The interference of diluent on analyte of interesting, was assessed by injecting the blank and the chromatogram is shown in Figure 1. The forced degradation studies were conducted to evaluate the stability-indicating capability and specificity of the proposed method [13,14]. Transferred 10 intact tablets in to 500 mL dried volumetric flask, added about 50 mL of methanol and sonicated for 15 minutes with intermittent shaking then 300 mL of diluent added and sonicated for 15 minutes with intermittent shaking. This solution is treated with 10 mL of 5N hydrochloric acid at 85 ^{o}C for 5 h, 10 mL of 5 N NaOH at 85 ^{o}C for 5 h and 10 mL of 30% H_2O_2 at 85°C for 3 h for acid, base and chemical oxidation degradations respectively. Before analysis acid and base treated solutions are neutralized and made up to the volume with diluent. Further these solutions are diluted to get 333 µg.mL^{-1}. In thermal degradation, tablets are subjected to dry heat at 105 ^{o}C for 5 days and analysed as per method. Peak purity test performed by the using of photo diode array detector to show the analyte chromatography peak did not contain more than one substance. Major degradation of Acamprosate is under acidic (12.20% degradation), alkaline (12.31% degradation) and oxidizing (29.6% degradation). Degradation under thermal was 2.2.

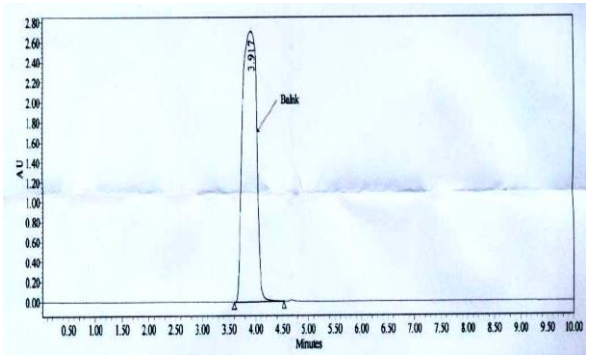

Figure 1. HPLC Chromatogram of blank

3.2. Linearity

Calibration curves were performed by analysis of working standard solutions of these formulations with at least six different concentrations in the range between 33 µg - 528 µg mL^{-1}. 33 µg mL^{-1} and 528 µg mL^{-1} concentrations were injected as six replicate injections. The retention time of Acamprosate is 4.685 min. The relation between Acamprosate concentration (*x*) and its corresponding peak area (*y*) i.e. expressed by the equation y=1581x+11367, with correlation coefficient of the calibration curve (*r*) 0.9999. The results show that within the concentration indicated there was an excellent correlation between peak area and concentration of each drug. Chromatograms of standard and sample are shown in Figure 2 and Figure 3.

3.3. Precision

Method precision (intra-day) was determined from results of six independent determinations of 100% of the test concentrations of Acamprosate Ca. Intermediate precision (inter-day) of the method

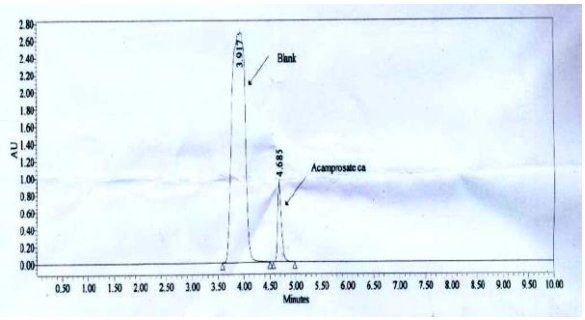

Figure 2. HPLC Chromatogram of standard

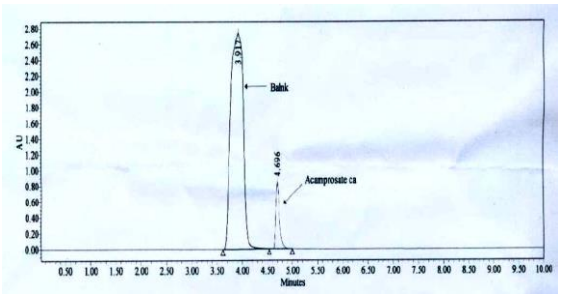

Figure 3. HPLC Chromatogram of sample

Table 1. Method precision

S.No	Intra day	Inter-day
1	100.4	100.2
2	100.2	101.0
3	99.9	100.4
4	99.3	99.5
5	99.6	100.7
6	100.2	100.5
Mean	99.9	100.4
SD	0.418	0.511
%RSD	0.42	0.51

was checked by different analyst and different day under the same experimental conditions. The relative standard deviation (RSD) was <0.51%. Both the results are summarized in Table 1. System precision was determined from results of six replicate injections of the standard solutions. The relative standard deviation was <0.2%.

3.4. Limit of Detection and Quantification

The limit of detection (LOD) and limit of quantification (LOQ) was estimated from the signal to noise ratio. This limit was defined as the lowest concentration level that provided a peak area with signal to noise ratio higher than 3:1 for detection and 10:1 for quantification [15]. The LOD and LOQ values for Acamprosate were 0.33 µg mL^{-1} and 1.0 µg mL^{-1} respectively.

3.5. Accuracy

Accuracy of method was performed at 20.0%, 100.0% and 160.0% of target concentration of analyte. Triplicate sets of samples at each concentration were prepared and injected by single injection into the liquid chromatography system and chromatograms were recorded. The accuracy was expressed as the percentage of analytes recovered by assay. Mean recoveries for Acamprosate Ca from the specific formulations are shown in Table 2. The results in Table 2 indicate that the method is highly accurate for the determination of Acamprosate.

Table 2: Accuracy

Level	mg added	mg found	Mean recovery	sd	% RSD
20 %	676.1	685.8	101.4	0.31	0.31
100 %	3295.7	3277.4	99.4	0.64	0.64
160 %	5241.9	5149	98.2	0.07	0.07

3.6. Robustness

The robustness of a method is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters because, the described method was designed for future application in the routine drug analysis by Pharmaceutical laboratories and other quality control laboratories. Robustness of the proposed method was evaluated by changing pH of the mobile phase (altered by±0.2 units), column temperature (altered by±5 ^{o}C), flow rate (altered by±0.1 mL/min), percentage of organic solvent (altered by±5%) and different phenyl columns (Phenomenex Luna Phenyl Hexyl, Zorbax SB Phenyl, Hypersil BDS Phenyl) of variation suppliers. The results indicated that there were no significant differences between the columns.

3.7. System Suitability

System suitability parameters were measured to verify the system performance and the retention time of Acamprosate was 4.685 min, theoretical plate numbers were above 3000 and the resolution between diluents peak and Acamproate is above 3.5 and tailing factor was below 2.0. All the values for the system suitability parameters were within an acceptable range.

3.8. Solution Stability

The stability of standard and sample solutions was determined by injecting every 6 hours for first 24 hours and 12 hrs interval for next two days, over a period of three days. The % assay of aged solutions was compared with those from freshly prepared standard solutions. The results showed that the retention times and peak areas of Acamprosate was with relative standard deviation of lower than 2% and that no significant degradation is observed within the given period, indicating that the solutions are stable.

3.9. Method Application

The HPLC method is rapid and sensitive for the quantitative determination of Acamprosate in their dosage forms. Acampral Tablets of Sun pharma were evaluated for the amount of Acamprosate present in formulation. The results indicate that the amount of drug in the tablets is within 98 to 102 % of the label claim.

4. CONCLUSIONS

The proposed method is suitable for the use of process quality control of Acamprosate Ca in commercial formulations with accurate and precise determination. In fact the reproducibility of the analysis is well below the 2.0% RSD and with a recovery of about 100.0%. Further to, it is rapid and highly specific method with less than 10 minutes run time and it allows the analysis of a large number of samples in short period of time. Although it is very simple, no interference from the other ingredients of the various examined formulations was observed.

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