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Original Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF ACAMPROSATE CALCIUM IN TABLETS

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ABSTRACT

Objective: To develop a cheap, accurate, precise, linear and rapid Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method and validate as per ICH guidelines for the quantitative estimation of Acamprosate calcium (333 mg) in tablets.

Methods: The optimized method uses a reverse phase column, Phenomenex Luna (250 X 4.6 mm; 5µ), a mobile phase of methanol:triethylammonium phosphate buffer (pH 3.0) in the proportion of 30:70 v/v, flow rate of 0.5 ml/min and a detection wavelength of 215 nm using a UV detector.

Results: The developed method resulted in Acamprosate calcium eluting at 5.68 min. Acamprosate calcium exhibited linearity in the range 16.65-58.275 µg/ml. The precision is exemplified by relative standard deviation of 1.02 %. Percentage Mean recovery was found to be in the range of 98-102, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 15 ng/ml and 45 ng/ml respectively.

Conclusion: A cheap, accurate, precise, linear and rapid RP-HPLC method was developed and validated for the quantitative estimation of Acamprosate calcium 333 mg in tablets as per ICH guidelines and hence it can be used for the routine analysis in various pharmaceutical industries.

Keywords: RP-HPLC, Acamprosate calcium, Method development, Validation.

INTRODUCTION

Acamprosate calcium (Figure 1, calcium 3-acetamidopropane-1-sulfonate) is the calcium salt of acetylhomotaurine used in the treatment of alcohol dependence. It is believed to act by blocking glutaminergic *N*-methyl-D-aspartate receptors and activation of gamma-aminobutyric acid (GABA) type A receptors [1-3]. 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 [4]. Acamprosate has been commercially available since 1989, in 333 mg tablet strength [5]. Acamprosate calcium is a white, odorless or nearly odorless powder. It is freely soluble in water and practically insoluble in absolute ethanol and dichloromethane. Its chemical formula is $C_{10}H_{20}N_2O_8S_2Ca$ and molecular weight is 400.48.

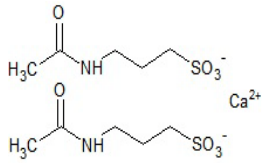

Fig. 1: Structure of Acamprosate calcium

A detailed literature survey reveals capillary zone electrophoresis methods [6-7], bioanalytical methods for the analysis of Acamprosate calcium using LCMS [8-16], LC-fluorometric and electrochemical detection [8] in human plasma, dog plasma and urine. Recently, one RP-HPLC method has been reported for the quantitative estimation of Acamprosate calcium in tablets [17]. We here report a new, cheap, precise, accurate and linear isocratic RP-HPLC method for the quantitative estimation of Acamprosate calcium in CAMPRAL tablets.

MATERIALS AND METHODS

Chemicals and Reagents

Analytically pure sample of Acamprosate calcium with purities greater than 99% was obtained as gift sample from Chandra labs, Hyderabad, India and tablet formulation [CAMPRAL] was procured from MEDPLUS, Hyderabad, India with labelled amount 333mg of Acamprosate calcium. Methanol (HPLC grade), water (HPLC grade), Triethylamine (AR Grade) and ortho phosphoric acid (AR Grade) were obtained from SD Fine chemicals (Hyderabad, India), 0.45µm Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad, India.

Instrument

HPLC analysis was performed on Shimadzu LC-20AD Prominence Liquid Chromatograph comprising a LC-20AD pump, Shimadzu SPD-20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Phenomenex Luna (250 X 4.6 mm; 5µ). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was controlled with "Lab solutions lite" software. An electronic analytical weighing balance (0.1mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (sonica, model 2200 MH) and UV-Visible Spectrophotometer (Shimadzu UV-1800 series, software-UV probe version 2.42) were used in this study.

Methods

Selection of Wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for Acamprosate calcium. Suitable wavelength selected was 215 nm (**Figure 2**).

Chromatographic conditions

The developed method uses a reverse phase C18 column, Phenomenex Luna (250 X4.6 mm; 5µ), mobile phase consisting of a mixture of methanol:triethylammonium phosphate buffer (adjusted to pH 3.0 with 30% v/v of ortho phosphoricacid) in the ratio of 30:70, v/v. The mobile phase was set at a flow rate of 0.5 ml/min and the volume injected was 20 µl for every injection. The detection wavelength was set at 215 nm.

Buffer Preparation

The buffer solution was prepared by adding 5 mL of triethylamine to 1000 ml of HPLC grade water and later pH was adjusted to 3.0 using

30% v/v of ortho phosphoric acid in water. The buffer was then filtered through 0.45 µm nylon membrane filter.

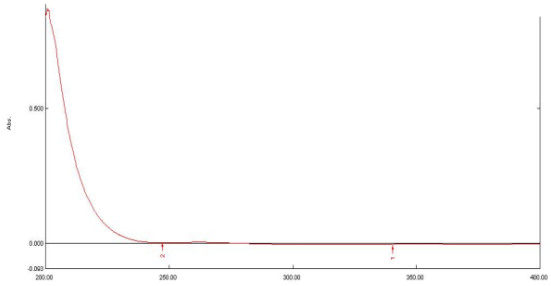

Fig. 2: UV spectrum of Acamprosate calcium

Mobile phase Preparation

The mobile phase was prepared by mixing methanol and buffer in the ratio of 30:70 v/v and later it was sonicated for 10 minutes for the removal of air bubbles.

Preparation of stock and working standard solution

33.3 mg of acamprosate calcium was accurately weighed and taken in 100 ml clean and dry volumetric flask containing 50 ml of diluent (same as mobile phase) and then sonicated for 5 minutes to dissolve. Later the solution was made up to the mark using the mobile phase. This is considered as standard stock solution (333µg/ml). 1 ml of the stock solution was pipetted out and made up to 10 ml to get a concentration 33.3 µg/ml, treated as working standard, 100% target concentration.

Preparation of stock and working sample solution

Ten tablets were weighed separately and the average weight was determined. The average weight was weighed from the ten tablets grinded in a pestle and mortar, transferred to a 100 ml volumetric flask containing 100 ml diluent and then stirred for 20 minutes, followed by filtration through 0.45µ nylon membrane filter to get sample stock solution of 3.33mg/ml. 1 ml of the above stock solution was pipetted out and made up to 100 ml to get working sample solution equivalent to a concentration of working standard of 33.3 µg/ml.

RESULTS AND DISCUSSION

Method Development

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of acamprosate calcium at 5.67 min. **Figures 3** and **4** represent chromatograms of blank solution and standard solution (33.3 µg/ml) respectively. The total run time is 7 minutes. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (*Rt*), number of theoretical plates (*N*) and peak Tailing factor (T) were evaluated for six replicate injections of the standard at working concentration. The results are given in Table 1.

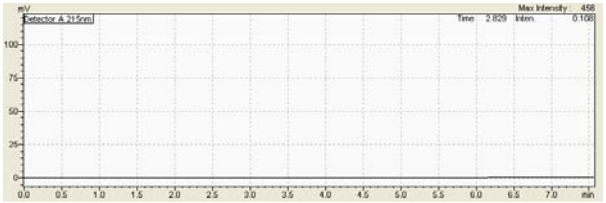

Fig. 3: Typical Chromatogram of Blank solution

In order to test the applicability of the developed method to a commercial formulation, 'CAMPRAL was chromatographed at working concentration and it is shown in Figure 5. The sample peak was identified by comparing the retention time with the standard drug **Figure 4**. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control.

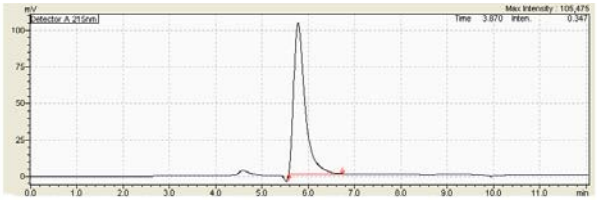

Fig. 4: Typical chromatogram of the standard solution

Table 1: System suitability studies results.

Parameters*	Acamprosate calcium
Retention time (min)	5.68
Number Of Theoretical plates (N)	7360
Tailing factor (T)	1.9

* Mean of six injections

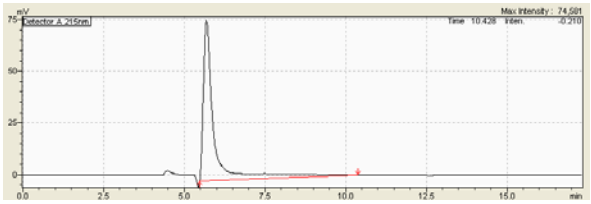

Fig. 5: Typical chromatogram for the tablet formulation

Method validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines [18] for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness, limit of detection (LOD) and limit of quantitiation (LOQ).

Specificity

Figures 3-5 for blank, standard drug solution and sample chromatogram reveal that the peaks obtained in the standard solution and sample solution at working concentrations are only because of the drugs as blank has no peak at the retention time of acamprosate calcium. Accordingly it can be concluded that, the method developed is said to be specific.

Precision

System precision

Six replicate injections of the standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning peak area for the drug, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in **Table 2**.

Method precision

Method precision was determined by performing assay of sample under the tests of (i) repeatability (Intra day precision) and (ii) Intermediate precision/ Ruggedness/ Inter day precision) performed during 3 consecutive days by three different analysts, at working concentration.

Repeatability (Intra day precision)

Six consecutive injections of the sample from the same homogeneous mixture at working concentration showed % RSD less

than 2 concerning % assay for the drug which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (Table 3).

Intermediate Precision (Ruggedness / Inter day precision)

Six consecutive injections of the sample solution from the same homogeneous mixture at working concentration on three consecutive days by three different analysts, showed % RSD less than 2 for % assay for the drug within and between days, which indicate the method developed is inter day precise / rugged (Table 4).

Table 2: System precision results

Injection number (n)	Acamprosate calcium Rt	Peak Area
1	5.671	1779840
2	5.677	1752221
3	5.698	1785574
4	5.678	1763258
5	5.687	1763772
6	5.690	1781349
Average		**1771002**
SD		**13133.79**
% RSD		**0.74**

Table 3: Intra day precision results

n	Acamprosate calcium % Assay
1	98.6
2	100.40
3	100.81
4	101.7
5	101.59
6	100.2
Average	**100.55**
S.D.	**1.034**
% R.S.D.	**1.02**

Table 4: Inter day precision results

n	% Assay of Acamprosate calcium		
	Day 1	Day 2	Day 3
1	98.6	100.1	99.5
2	100.40	101.69	99.68
3	100.81	101.7	98.6
4	101.7	100.56	100.02
5	101.59	100.3	100.12
6	100.2	99.6	99.43
Average	100.55	100.65	99.55
SD	1.034	0.86	0.54
% RSD	1.02	0.85	0.54

Table 5: Linearity of the chromatography system

Drug	Linearity range (µg/ml)	R^2	Slope	Intercept
Acamprosate calcium	16.65-58.27	0.998	44406.713	267239.9

Table 6: Calibration data for Acamprosate calcium

% Level	Concentration (µg/mL)	Peak Area
50	16.65	1023431
75	25.97	1405827
100	33.3	1752913
125	41.62	2112631
150	49.95	2442952
175	58.27	2890945

Linearity

Standard solutions of acamprosate calcium at different concentrations level (50%, 75%, 100%, 125%, 150% and 175%) were prepared. Calibration curve was constructed by plotting the concentration level of drug versus corresponding peak area. The results show an excellent correlation between peak area and concentration level of drug within the concentration range (16.65-58.27 µg/ml) for the drug and the results are given in Tables 5-6 and Figure 6. The correlation coefficient of acamprosate calcium is 0.998, which meet the method validation acceptance criteria and hence the method is said to be linear.

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (50-150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in Table 7. The accepted limits of recovery are 98% - 102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Sensitivity

The sensitivity of measurement of acamprosate calcium by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 15ng/ml and 45 ng/ml.

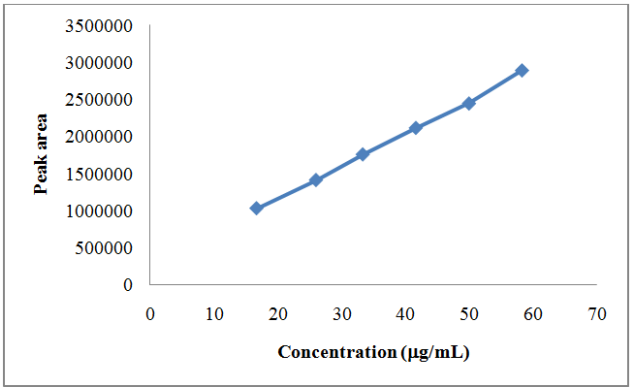

Fig. 6: Calibration curve for Acamprosate calcium.

Table 7: Results of Accuracy studies for Acamprosate calcium

Concentration Level (%)	*%Mean Recovery
50	100.95
100	100.4
150	101.13

*Mean of three replicates

CONCLUSION

A reverse phase HPLC isocratic method developed has been validated as per ICH guidelines in terms of specificity, accuracy, precision, linearity, ruggedness, limit of detection and limit of quantitation, for the quantitative estimation of acamprosate calcium in tablets. A good linear relationship was observed for the drug between concentration ranges of 16.65 and 58.27µg/ml. The inter day and intraday precision results were good enough to say that the method developed is precise and reproducible. Accuracy studies revealed that mean recoveries were between 98 and 102%, an indicative of accurate method. Accordingly it can be concluded that the developed reverse phase isocratic HPLC method is accurate, precise, linear and rugged and therefore the method can be used for the routine analysis of acamprosate calcium in tablets.

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CONFLICT OF INTERESTS

Declared None

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