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Original Article

UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF ACAMPROSATE CALCIUM IN TABLETS

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ABSTRACT

Objective: To develop a rapid UV spectrophotometric method for the quantitative estimation of Acamprosate calcium (333mg) in tablets and validate as per ICH guidelines.

Methods: The optimized method uses a diluent 100% Triethylammonium phosphate buffer (pH 4.0) for the estimation of assay of Acamprosate calcium at a detection wavelength of 208 nm.

Results: The developed method resulted in Acamprosate calcium exhibiting linearity in the range 30-90µg/ml. The precision is exemplified by relative standard deviation of 1.5%. 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) were found to be 99ng/ml, and 300ng/ml respectively.

Conclusion: A rapid UV spectrophotometric method was developed and validated for the quantitative estimation of Acamprosate calcium in tablets as per ICH guidelines and hence it can be used for the routine analysis in various pharmaceutical industries.

Keywords: UV, 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.

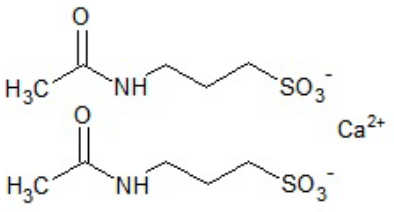

Fig. 1: Structure of Acamprosate calcium

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 and overall only one UV method has been reported for the quantitative estimation of Acamprosate calcium in tablets [17]. We here report a new, precise, accurate and linear UV method for the quantitative estimation of Acamprosate calcium in ACAMPRAL tablets.

MATERIALS AND METHODS

Materials

Instrument

A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path and loaded with UV probe software (version 2.41) was used for recording of spectra and measuring absorbance. An electronic analytical weighing balance (0.1mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101) and a sonicator (sonica, model 2200 MH) were used in this study.

Chemicals and Reagents

Analytically pure sample of Acamprosate calcium with purities 95% was obtained as gift sample from Chandra labs, Hyderabad, India and tablet formulation [ACAMPRAL] was procured from MEDPLUS, Hyderabad, India with labelled amount 333mg of Acamprosate calcium. Triethylammonium phosphate(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.

Method

Solvent

Solvent used is prepared by adding 5ml of triethylamine to 1000 ml of distilled water and later pH was adjusted to 4.0 using 30% v/v of ortho phosphoric acid in water. Solvent was then filtered through 0.45 µm nylon membrane filter.

Selection of suitable detection wavelength

Suitable wavelength for the total experiment was determined by recording UV spectrum in the range of 200-400 nm for Acamprosate calcium and suitable wavelength selected was 208 nm (Figure 2).

Preparation of stock and working standard solution

10mg of Acamprosate calcium was accurately weighed and taken in 100ml clean and dry volumetric flask containing 80ml of solvent and

then the solution was made up to the mark using the solvent. This is considered as standard stock solution (100µg/ml). 6ml of the stock solution was pipetted out and made up to 10 ml to get a concentration 60µg/ml, treated as working standard, 100% target concentration.

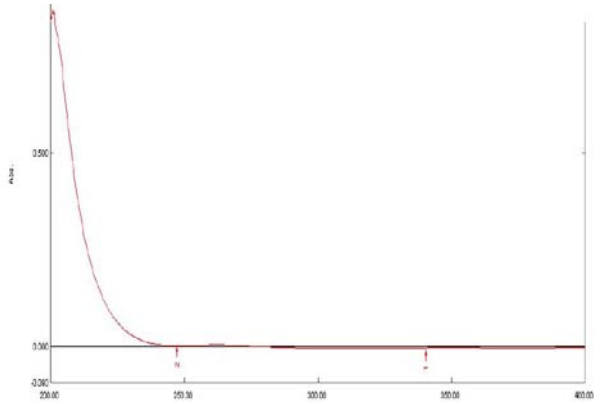

Fig. 2: UV spectrum of Acamprosate calcium

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 100ml diluent and then sonicated for a minute, followed by filtration through 0.45µ nylon membrane filter to get sample stock solution of 3330µg/ml. 1.8 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 60 µg/ml.

RESULTS AND DISCUSSION

Method Development

Various solvents were explored, including Potassium dihydrogen orthophosphate, triethylammonium phosphate and ammonium acetate buffers varying pH in the ranges of 2-7. Acamprosate calcium was found to be soluble and stable for minimum of 1 hour at room temperature using pH 4.0 triethylammonium phosphate buffer and hence this buffer was initiated for the determination of suitable detection wavelength and working concentration of standard. In order to test the applicability of the developed method to a commercial formulation, ACAMPRAL was studied at working concentration. Absorbance and assay for working concentration of sample at 208 nm was in acceptance limits (98-102%) with the standard working concentration during extraction of drug in the sample using the solvent. The protocol affords reproducible quantification of the drug in the sample ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control. Hence the method is optimized.

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. UV spectrophotometric 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 linearity, accuracy, system precision, intra-day precision, inter-day precision/ intermediate precision/ ruggedness, robustness, limit of detection (LOD) and limit of quantitiation (LOQ).

Precision

System precision

Six replicate recording of absorbance at 208nm of standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning absorbance for the drug, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in Table 1.

Method precision

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

Table 1: System precision results of Acamprosate calcium.

n	Absorbance
1	0.332
2	0.335
3	0.331
4	0.329
5	0.332
Average	**0.332**
SD	**0.002**
% RSD	**0.6**

Repeatability (Intra day precision)

Six consecutive recording of absorbance at 208nm 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 2**).

Table 2: Intra day precision results of Acamprosate calcium

n	% Assay
1	100.92
2	98.21
3	99.45
4	102.15
5	100.43
Average	**100.23**
S.D.	**1.490**
% RSD	**1.486**

Intermediate Precision (Inter day precision / Ruggedness)

Six consecutive recording of absorbance at 208nm 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 3)**.

Table 3: Inter day precision results.

n	Day 1	Day 2	Day 3
1	100.92	101.17	98.01
2	98.21	100.23	101.06
3	99.45	99.26	102.00
4	102.15	102.00	99.56
5	100.43	98.76	101.85
Average	**100.23**	**100.28**	**100.49**
SD	**1.490**	**1.332**	**1.69**
% RSD	**1.486**	**1.32**	**1.68**

Linearity

Standard solutions of Acamprosate calcium at different concentrations level (50%, 75%, 100%, 125%, 150%, 175% and 200%) were prepared. Calibration curve was constructed by plotting

the concentration level of drug versus corresponding absorbance at 208nm. The results show an excellent correlation between absorbance and concentration level of drug within the concentration range (30-90µg/ml) for the drug and the results are given in **Table 4.** The correlation coefficients were greater than 0.995, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 30-90µg/ml.

Table 4: Calibration data for Acamprosate calcium

% Level	Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3
50	30	0.202	0.198	0.210
75	45	0.301	0.312	0.301
100	60	0.400	0.409	0.407
125	75	0.505	0.513	0.510
150	90	0.609	0.605	0.608
Regression equation		y=0.101x+0.099	y=0.101x+0.102	y=0.100x+0.105
Regression coefficient		0.9996	0.9987	0.9994

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 5.** 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.

Table 5: Results of Accuracy studies for Acamprosate calcium

Concentration level (%)	*%Mean recovery (% RSD)
50	99.04(1.25)
100	100.26(0.74)
150	100.07(0.519)

*Mean of three replicates

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is concluded that the method is robust as it is found that the % RSD is less than 2 for the drug concerning % assay despite deliberate variations done concerning pH ± 0.2 and detection wavelength ± 2nm (Table 6).

Table 6: Robustness results of Acamprosate calcium sample

Variation parameter	Variation	%RSD
pH(± 0.2)	4.2	1.26
	4	0.948
	3.8	1.35
Wave length (± 2nm)	210	1.58
	208	1.80
	206	1.38

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 limit of detection (LOD). The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 99ng/ml, and 300 ng/ml respectively. Optical characteristics and validation parameters results are summarized in Table 7.

Table 7: Optical characteristics and validation parameters of Acamprosate calcium

Parameters	Results
Detection wavelength (nm)	208
Beer's Law limits (µg/ml)	30-90
Regression equation (y = mx+c)	y=0.100x+0.102
Correlation coefficient (r^2)	0.9992
Slope (m)	0.100
Intercept (c)	0.102
% Relative Standard Deviation (% RSD) System precision	0.473
(% RSD) Intra-day precision	1.486
(% RSD) Inter-day precision	≤2
Accuracy (% Mean Recovery)	
50 % Level	99.04 %
100 % Level	100.26 %
150 % Level	100.07 %
LOD (µg/ml)	0.099
LOQ (µg/ml)	0.300
Robustness	
pH(± 0.2) (% RSD)	≤2
Wavelength (± 2nm) (% RSD)	≤2

CONCLUSION

A rapid UV spectrophotometric method was developed and validated for the quantitative estimation of Acamprosate calcium in tablets as per ICH guidelines. The developed method resulted in Acamprosate calcium exhibiting linearity in the range 30-90 µg/ml. The precision is exemplified by relative standard deviation of 1.5%. 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) were found to be 99ng/ml and 300ng/ml respectively. Accordingly it is concluded that the developed UV spectrophotometric method is accurate, precise, linear, rugged and robust and therefore the method can be used for the routine analysis of Acamprosate calcium in tablets in various pharmaceutical industries.

CONFLICT OF INTERESTS

Declared None

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