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Validated UV Spectrophotometric Method Development And Stability Studies Of Acamprosate Calcium In Bulk And Tablet Dosage Form

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Abstract: A simple, rapid, accurate, precise, specific and economical spectrophotometric method for estimation of Acamprosate calcium in tablet dosage form has been developed. Beer-Lambert's law was followed in the concentration range of 10-50µg/ml (r^2= 0.999). LOD and LOQ were found to be 0.3626µg/ml & 1.0989µg/ml respectively. The result of percentage recoveryand placebo interference shows that the method was not affected by the presence of common excipients. Thepercentage assay ofAcamprosate calcium in tabletswas 99.4%w/w.The method was validated by determining its sensitivity, accuracyand precision which proved suitability of the developed method for the routine estimation of Acamprosate calciumin solid dosage form and it was subjected to stress degradation under different conditions recommended by ICH.
Keywords: Acamprosate calcium, Stability studies, UV method, Tablet dosage form.

1. Introduction[1-7]:

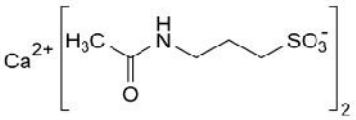

Fig 1: Molecular structure of Acamprosate calcium

Acamprosate calcium, Calciumbis[3-(acetyl amino)propane-1-sulphonate] is used in the treatment of Alcohol addicted patients.The drug is official in European Pharmacopoeia.Literature survey reveals thatfew analytical methods like Capillary electrophoresis, HPLC, LC/MS/MS, GC/MSwere reported. Herein efforts are made on development of simple UV-spectrophotometric method using phosphate buffer of pH 6.8 as vehicle.

2. Experimental[1-7] :

2.1. Materials and Methods:

ShimadzuUV-Visible Spectrophotometer-1800 with 1cm matched quartz cells were used for all spectral measurements. All chemicals were obtained fromLobhachemie and Merck Pvt.Ltd, Mumbai, unless otherwise

specified doubled distilled water was used to prepare all solutions. Sample standard Acamprosate calcium bulk drug was obtained from Mylan Laboratories and was systematically authenticated for its standard and identity.

2.2. Preparation of standard stock solution:

An accurately weighed 10mg of Acamprosate calcium was taken and dissolved in 10ml of phosphate buffer (pH6.8) in a 100ml volumetric flask and the volume was adjusted up to the mark with distilled water to get a concentration of 100µg/ml and it is named as stock 1. From the stock 1, various working concentrations were prepared by further dilutions with same solvent. The solutions were scanned on spectrophotometer in UV range 200-400nm. Acamprosate calcium showed absorbance maxima at 205nm. The scanned spectrum was showed in Fig 2.

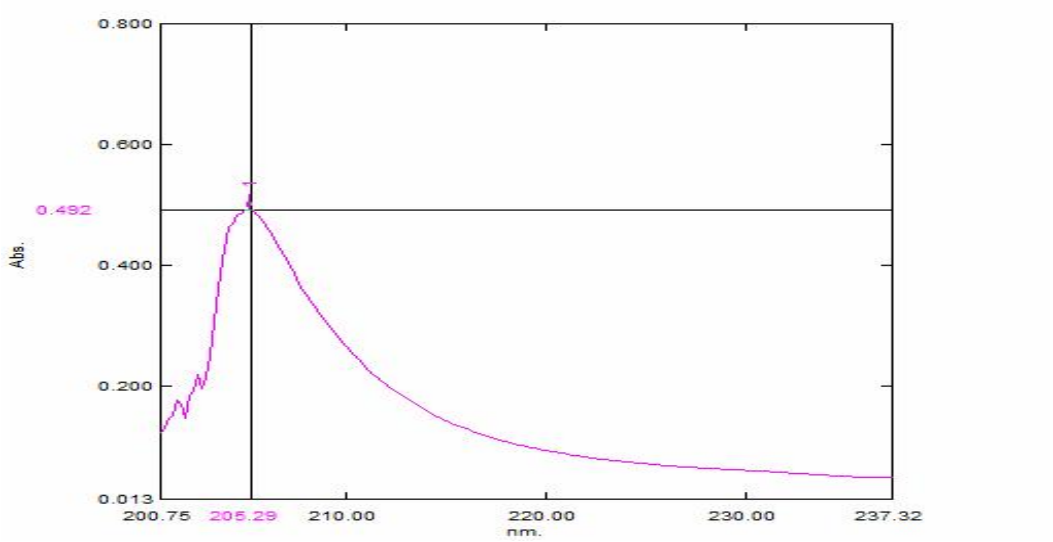

Fig. 2: UV-spectrum of Acamprosate calcium in phosphate buffer (pH 6.8)

2.3. Preparation of Calibration Curve:

Stock solution 1 was further diluted with phosphate buffer (pH 6.8) to get working concentrations ranging from 10-50µg/ml. The calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis. The curve showed linearity with correlation coefficient (r^2) 0.999, shown in fig 3.

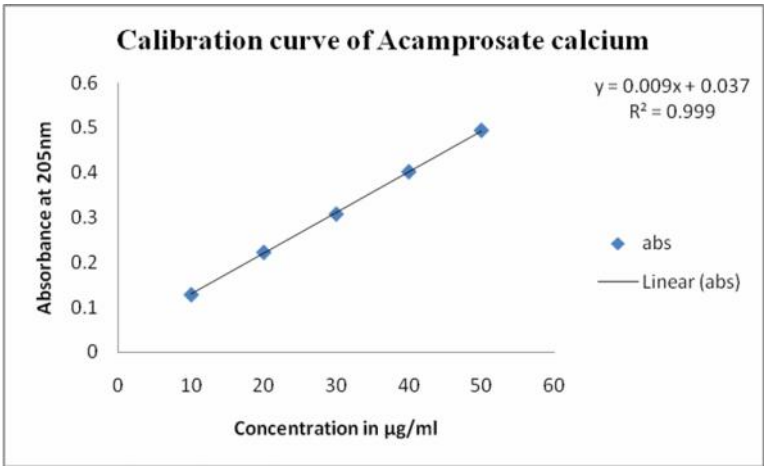

Fig 3: Calibration curve of Acamprosate calcium

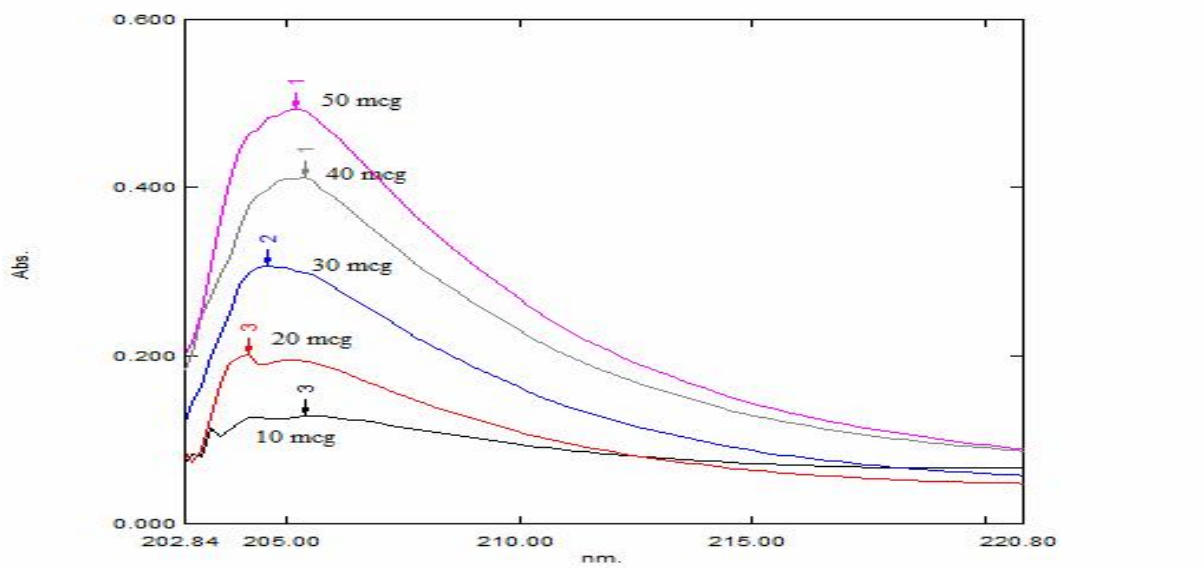

Fig 4: UV-spectrum of Acamprosate calcium (10-50µg/ml) with phosphate buffer (pH 6.8)

3. Method Validation:

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. Method was validated for different parameters like Linearity, Precision, Limit of Detection (LOD) and Limit of Quantification (LOQ).

3.1. Linearity:

The method was validated according to ICH Q2Bguidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy of the analyte. Eight point calibration curves were generated with appropriate volumes of the working standard solutions for UV methods. The linearity and regression equation was shown in Fig 3.

3.2. Precision:

Precision is the degree of repeatability of an analytical method under normal operational conditions. Both Inter-day precision and Intra-day precision were carried out as per the statistical requirement to support reproducibility of the method. The results were shown in Table 2.

3.3. LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations LOD=3s/m; LOQ=10s/m. Where s, the noise of estimate, is the standard deviation of the absorbance of the sample and m is the slope of the related calibration graphs. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision, and variability. The values of LOD and LOQ were given in table 1.

3.4. Recovery

Recovery of the analyte of interest from a given matrix can be used as measure of the accuracy or the bias of the method. The same range of concentrations as employed in the linearity studies was used. Recovery studies was carried out on standard addition of 80%, 100%, 120% of labeled dose and the total amounts were determined by the method and the %recovery reports were shown in table 3.

4. Degradation Studies:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Acamprosate calcium using the method developed. The results of Stability studies were shown in Table 4.

Table 1: Summary of Validation parameters

S. No.	Parameter	Result
1	λ_{max}(nm)	205
2	Beer-Lambert's law range (µg/ml)	10-50
3	Regression equation a.Slope b.Intercept c. Correlation co-efficient	Y=0.009x+0.035 0.009 0.035 0.999
4	Limit of detection (LOD, µg/ml)	0.3626
5	Limit of quantification (LOQ, µg/ml)	1.0989
6	Precision indicated by %RSD	0.9583
7	% Recovery	99.4%

Table 2: Results of both Intra-day and Inter-dayPrecision

Sample (Acamprosate calcium)	Days	concentration	Absorbance*	%RSD
Intra-day precision	1	10 µg/ml	0.126	1.7770
		30 µg/ml	0.334	0.9583
		50 µg/ml	0.493	0.4333
Inter-day Precision	1	30 µg/ml	0.335	0.6965
	2		0.335	0.5576
	3		0.335	0.8715

*mean of six determinations

Table 3: Recovery readings of Acamprosate calcium

S.no	Concentration		%Recovery	Mean	SD	%RSD
	Formulation	Pure drug				
1	10	8 (80)	101.4	100.7	0.68505	0.68
2	10	10 (100)	99.4	100.4	0.95393	0.95
3	10	12 (120)	100.4	99.95	0.45014	0.45

Table 4: Stress degradation studies

S. No.	Stress condition	Concentration (µg/ml)	Strength	Absorbance at 205nm
1.	HCl	30	1N	0.194
			2N	0.114
			3N	0.153
2.	NaOH	30	1N	0.154
			2N	0.360
			3N	0.201
3.	H_2O_2	30	10%	-0.783
			20%	2.851
			30%	2.417
4.	UV	30	30min	0.348
			60min	0.401
5.	Dry Heat	30	80^{o}C for 1hr	0.417

4.1. Effect of pH:

4.1.1. Study in acidic condition: From stock solution (100µg/ml) 3ml was taken into 10ml flasks and volume was made up with increasing normality strengths of HCl to get a final concentration of 30µg/ml and absorbance was measured at 205nm using appropriate diluents as blank.

4.1.2. Study in alkaline condition: From stock solution (100µg/ml) 3ml was taken in to 10ml flasks and volume was made up with increasing normality strengths of NaOH to get a final concentration of 30µg/ml and absorbance was measured at 205nm using appropriate diluents as blank.

4.2. Effect of Oxidation (Hydrogen Peroxide): From stock solution (100µg/ml) 3ml was taken in to 10ml flasksand volume was made up with 10%, 20% and 30%H_2O_2 to get 30µg/ml concentration and absorbance was measured at 205nm using appropriate diluents as blank.

4.3.Effect of UV Light (Photo Stability): From stock solution (100µg/ml) 3ml was taken in to 10ml flasks and volume was made up with diluents (Phosphate buffer pH 6.8) to get 30µg/ml and absorbance was measured at 205nm at different time intervals in UV cabinet.

4.4.Effect of Dry Heat: Acamprosate calcium sample was taken in a petri plate and exposed to a temperature of 80°C for 1 hour in an oven. After 24 hours, 10mg of the sample was diluted with Phosphate buffer (pH 6.8) to make the volume up to 100ml. From this solution, dilution was carried out to achieve the appropriate concentration (30µg/ml) and the solution absorbance was measured at 205nm.

5. Results And Discussion:

Acamprosate calcium shows λ_{max} at 205nm and the linearity plot yielded a correlation coefficient (r^2)of 0.999 over the Beer-Lambert's range of 10-50µg/ml. The regression equation was found to be Y=0.009x+0.035. The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. The LOD and LOQ were found to be 0.3626&1.0989µg/ml respectively. The stress degradation studies showed that Acamprosate calcium undergoes degradation in increased strengths of acidic, alkaline, oxidation (Hydrogen peroxide). It is relatively stable in photolytic conditions and dry heat. The proposed UV method was found to be simple, sensitive, selective, accurate, precise and economical which can be used in the determination of Acamprosate calcium in bulk and in tablet dosage form.

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