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Spectrophotometric Determination of Cycloserin in Bulk and Capsule Dosage form by Area Under Curve and First Order Derivative Methods

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Abstract : It is an simple, precise and economical UV- spectrophotometric method has been developed for the estimation of Cycloserinein pharmaceutical dosage form. First method (A) applied was area under curve (AUC) in which area was integrated in the wavelength of range212.0nm-222.0 nm. Second method (B) was first order derivative spectrometric method. In which method absorbance at λmin =207.6nm, λmax = 236.4 and zero cross= 217.0 nm was measured. The calibration curves were plotted for the method by using instrumental response at selected wavelengths and concentrations of analyte in the solution. Linearity observed in the concentration range of 5-25 µg/ml for the method. Capsule formulation was analyzed and the percentage of drug determined in the assays was 98.00-99.00%. Accuracy and precision studies were carried out and results were satisfactory obtained. The results of the analysis were validated statistically. Limit of detection (LOD) and limit of quantitation (LOQ) were determined for the method. The method was validated by the International Conference on Harmonization. All validation parameters were within the acceptable limit. The developed method was successfully applied to estimate the amount of Cycloserine in pharmaceutical formulation.

keywords: Cycloserine, UV-spectrophotometry, Area Under Curve, First order derivative, Validation.

Introduction:

Cycloserine is a broad spectrum antibiotic. Cycloserine is an analog of amino acid D-alanine. It inhibits alanine racemase and D-alanine, D-alamine ligase which synthesizes the pentapetide core using D-alanine both enzymes are essential in the synthesis of peptidoglycan and subsequently in cell-wall synthesis and maintains. [1] It is most often used in combination with up to 5 drugs to treat. Cycloserine may be bactericidal or bacteriostatic, depending on local concentration effect as well as efficacy against the particular strain involved. [2,3]

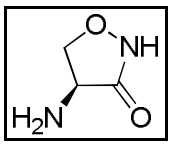

Fig. 1 Structure of cycloserine.

Chemically cycloserine is D-4-Amino-3-isoxazolidone as shown in Fig. 1. Molecular formula is $C_3H_6N_2O_2$ and it is molecular weight 102.09.[4,5] Cycloserine which melting point on 147°C, it is white or pale yellow colour which is slightly hygroscopic in nature and degrade upon expose to humid atmosphere, So cycloserine should be kept in tightly closed container. [6,7]

Materials and Methods:

Apparatus and Instrumentation

Shimadzu UV 1800 (Japan) with matched quartz cells, connected to computer loaded with UV Prob Software. Single pan electronic balance (Shimadzu, ATY 224) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonication (Spectralab UCB 40, India). Calibrated volumetric glasswares (Borosil) were used in this study.

Materials

Capsule of 250 mg strength were purchased from the local pharmacy in Pune under commercial available brand name CyclorinR (Lupin Laboratories Ltd.). Hydrochloric acid purchased from Loba Chemie Pvt Ltd, Mumbai (for preparation of 0.01N HCl). Double distilled water was used in this study.

Method development

Preparation of Standard Solution

The standard stock solution of cycloserine was prepared by transferring accurately weighed 100 mg of cycloserine to 100 ml volumetric flask containing 10 ml 0.01N HCl. Dissolve drug properly. Then volume was made up to the mark by using 0.01N HCl to gives concentration 1000 µg/ml. From this 10 ml of the solution was transferred to a 100 ml volumetric flask and make up the volume with0.01N HCl to gives a concentration of 100 µg/ml which is standard stock solution and it is further diluted with 0.01 N HCl to get concentration 5-25 µg/ml.

Selection of wavelength range

The working standard solution of 15 µg/ml was scanned between 400 nm to 200 nm in UV spectrophotometer against 0.01N HCL as blank after baseline correction. Wavelength range selected around wavelength maxima 217.0 nm concentration (Figure 2).

Area under curve (Area calculation)

This method involves calculation of integrated value of absorbance with respect to wavelength in indicated range. Area calculation processing it emcalculates the area bounded by the curve and horizontal axis. Here horizontal axis represents baseline.

$$Area\ calculation\ (\alpha+\beta)=\frac{\lambda_1}{\lambda_2}Ad\lambda$$

Whereas, α is area of portion bounded by curved at a and a straight line connecting the start and end point, β is area of portion bounded by a straight line connecting the startand end point on curve data and horizontal axis, λ_1 and λ_2 are wavelenght srepresenting start and end point of curve region. In this study area was integrated between wavelength ranges from212.0- 222.0 nm.

First Order Derivative Spectrophotometry (Method B):

In this method solution of Buspirone HCL (5-25 µg/ml) were separated by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The absorption spectra obtained were derivatised from first fourth order. First order derivative spectra were selected for analysis of drug. From spectra of drug the absorbance was measured at λmax=207.6 nm, λmin=236.4.0 nm and zero cross =217.0nm, amplitude difference (dA) with respect to wavelength difference ($d\lambda$) was measured for the

respective concentration of standard and was plotted against concentrations and regression equation was calculated.

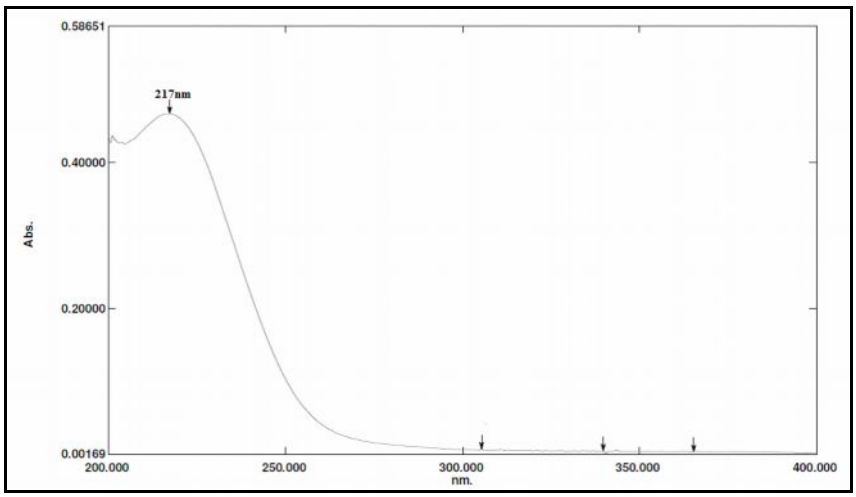

Figure 2: Determination of λmax Cycloserine

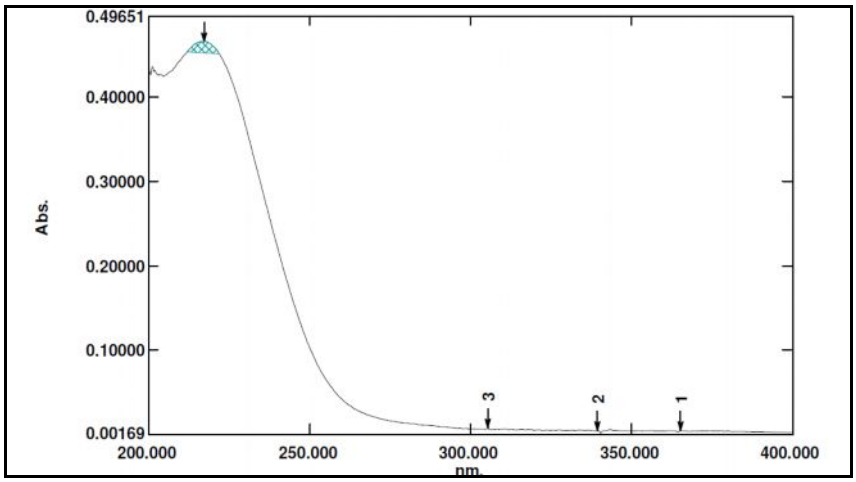

Figure 3: Area under curve graph of 15 µg/ml Cycloserine solution

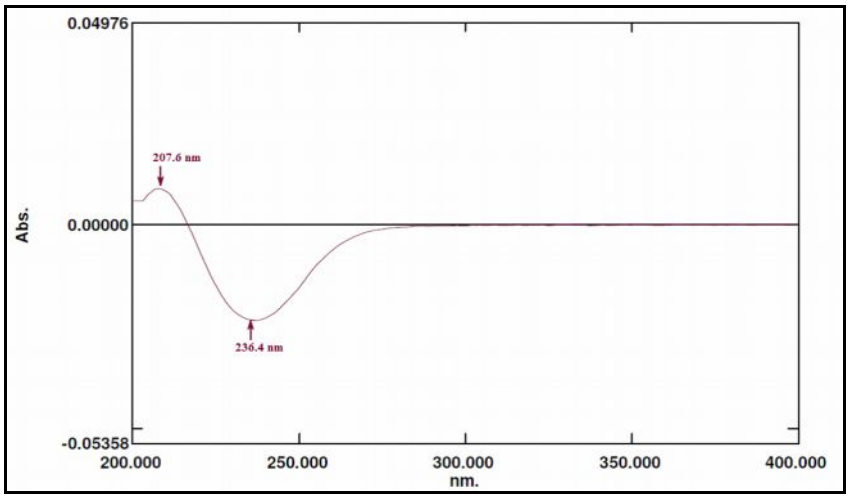

Figure 4: First order derivative spectra of Cycloserine

Preparation of calibration curve

Working solutions were prepared from standard stock solution by further dilution with methanol to obtain the concentration of 5, 10, 15, 20 and 25µg/ml, respectively. The solutions were scanned from 400 to 200 nm and area under curve (AUC) was integrated in the range of 212-222 nm. The calibration curve was plotted between area under curve against concentration(Figure 3).

Assay of capsule formulation

Twenty capsules were weighed accurately, open the capsule shells and remove the powder. Powder equivalent to 10 mg cycloserine was weighed and transferred to a 100 ml volumetric flask. It was dissolved in 100 ml 0.01N HCl and sonicated for 5 minutes to get homogeneous solution. Then it was first filtered through a 0.45µm whatman filter paper. A final concentration of 100 µg/ml of cycloserine was prepared. This solution was filtered through filter paper to remove some un-dissolved excipients. After filtration, from this 1.5 ml was taken and diluted to 10 ml with Distilled water which gives 15µg/ml solution This procedure repeated three times (Table 1).

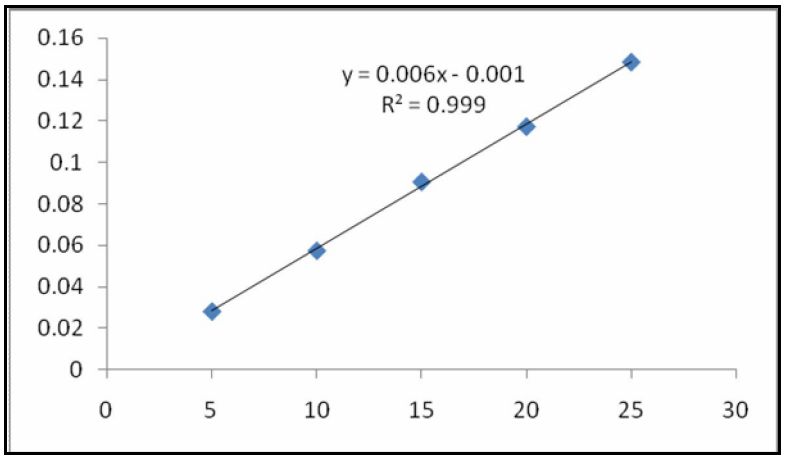

Figure 3: Calibration curve AUC of Cycloserine

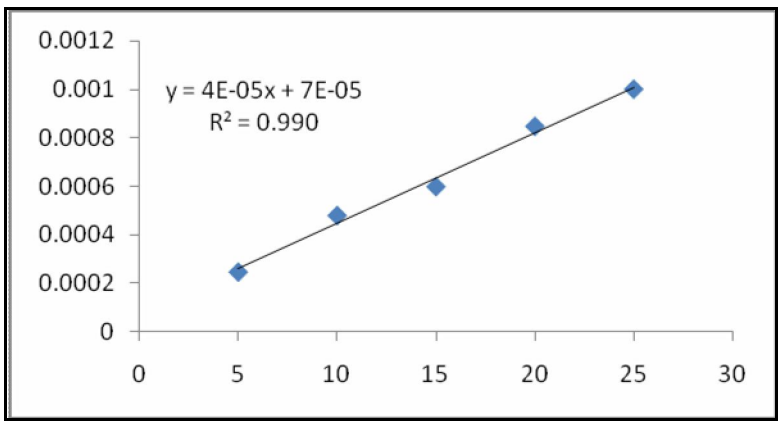

Figure 4: Calibration curve first order derivative of Cycloserine

Table 1: Assay of Marketed Formulation of Cycloserine

Method	Label claim	Amount taken	Amount found (mg/cap)	Assay %
A	250 mg	10mg	9.853 mg	98.53%
B	250 mg	10 mg	9.812 mg	98.12%

Table 2:Calibration data of Cycloserine

Concentration(µg/ml)	Method A (Area under curve)	Method B (First order derivative)
5 µg/ml	0.02822	0.000246
10 µg/ml	0.05745	0.000479
15 µg/ml	0.09061	0.000597
20 µg/ml	0.11718	0.000846
25 µg/ml	0.14829	0.000999

Table 3: Result of precision

Precision	Method A(%RSD)	Method B (%RSD)
Repeatability	0.7654	0.3021
Intraday	0.5664	0.7685
Interday	0.9835	0.6839

In method A, the concentration of Cycloserin was determined by measuring area under curve in the range of 212.00nm to 222.00nm and values were substituted in the respective formula to obtain concentration.

In method B first order derivative spectrophotometry the concentration of solution determined by measuring absorbance at λ min= 207.60nm, λmax=236.4nm and zero cross=217.00nm.This procedure was repeated in triplicate.

Table 4: Recovery Study of Cycloserine

Test(µg/ml)	Accuracy level	Amount of standard drug added (µg/mL)	Method A % Recovery	Amt. of Drug Std. Added (µg)	Method B % Recovery
15µg/ml	80%	12	99.94	12	98.90
	100%	15	99.56	15	99.76
	120%	18	98.03	18	99.56

Table 5: LOD and LOQ of Cycloserine

Methods	Method A	Method B
LOD	0.08966	0.4919
LOQ	0.2716	1.4907

Method validation

The objective of validation of ananalytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity, Accuracy, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline.[8]

Linearity and Range

The linearity was determined by using working standard solutions between 05-25µg/ml. The spectrums of these solutions were recorded and area under curve was integrated in wavelength range 212-222 nm. Calibration curve of Area under curve versus Concentration was plotted after suitable calculation and simple linear regression was performed (Figure3). Regression equation and correlation coefficient were obtained. The range of solution has been decided according to statistical parameters of generated equation.

Method Precision

Repeatability

The precision of the method was checked by repeatedly injecting (n=6) standard solutions of Cycloserine (15 µg/mL). Area under curve of each of these solutions was measured in the range of 212-222 nm. Percentage relative standard deviation (%RSD) was calculated (Table 3).

Intermediate Precision (Reproducibility)

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of Cycloserine (5, 10 and15µg/mL).The results were reported interms of relative standard deviation (%RSD).The results were tabulated in (Table 3).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Six sets of known concentrations (5-25 µg/ml) were prepared. Calibration curves were plottedfor each set. LOD and LOQ were calculated using the formulae as:

$$LOD = 3.3\ \frac{SD}{S}$$

$$LOQ = 10\ \frac{SD}{S}$$

Where,SD is standard deviation of y-intercept of the calibration curves, S is mean slope of six calibration curves.

Accuracy

The accuracy for the analytical procedure was determined at 80%, 100% and120% levels of standard solution. Area under curve was measured in the range of 212-222 nm and results were expressed interms of % recoveries. Three determinations at each level were performed and% RSD was calculated. The results were tabulated in (Table 4).

Results and discussion

An attempt was made to develop a simple and specific AUC spectrophotometric method for the determination of cycloserine in capsule dosage form. The generated regression equation was

$$\int_{212}^{222} Ad\lambda = 0.006x - 0.0016 \qquad R^2 = 0.9992$$

Where, $\int_{212}^{222} Ad\lambda$ is area under curve between 212 to 222 nm, C is concentration and R^2 is correlation coefficient. The R^2 value as 0.9992 indicate that developed method was linear. The proposed method was found tobe precise as % R.S.D values for intraday as well interday precision were satisfactory. The drug at each of the 80%, 100% and120% levels showed good recoveries (98% to 101%). Hence, it can be said that this method was accurate. The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The method can be used for the routine analysis of the Cycloserine in capsule dosage form. The validation parameters are summarized in (Table 6).

Table 6: Summary of Cycloserine

Parameter	Method A	Method B
Rang	212.0-222.0	310nm
Linearity range	5-25 µg/ml	5-25 µg/ml
Regression equation ($y = mx + c$)	0.006x - 0.0016	4E-05x + 7E-05
Slope (m)	0.0016	7E-05
Intercept (c)	0.006	4E-05x
Correlation coefficient (R^2)	0.9992	0.9908
Repeatability	0.7654	0.3021
Intraday	0.5664	0.7685
Interday	0.9835	0.6839
Accuracy (Mean % Recovery)	99.18	99.41
LOD	0.08966	0.4919
LOQ	0.2716	1.4907

Results and Discussion

An attempt was made to develop a simple and specific AUCand first order derivative spectrophotometric method forthedetermination of Cycloserine in capsuledosage form. The generated regression equations were,

Method A - $\int_{212}^{222} A\,d\lambda$ 0.006x-0.0016 R^2=0.9992

Method B – $\frac{dA}{d\lambda}$ 4E-05x+7E-05

Where $\int_{212}^{222} A\,d\lambda$ is area under curve between 212.00 nm – 222.00 nm, $\frac{dA}{d\lambda}$ is amplitude difference, x is concentration and R2is correlation coefficient. The R2 values were 0.9992 and 0.9908 for Method A and B respectively indicated thatdeveloped methods were linear. The proposed methodswere found to be precise as % RSD values for intraday aswell as interday precision were satisfactory. The drug at each of the 80 %, 100 % and 120 % levels showed goodrecoveries that is in the range of 98.00to 99.00%, hence itcould be said that these methods were accurate. The LODand LOQ were calculated as 0.08966 µg/ml and 0.2716µg/ml for method A and 0.4917µg/ml and 1.4907µg/ml formethod B respectively. The results of analysis ofpharmaceutical formulation by the developed methodswere consistent with the label claim, highly reproducibleand reliable and hence the methods can be used for the routine analysis of the Cycloserinein capsuledosage forms. The validation parameters for method A andmethod B are summarized in Table 6.

Conclusion

The two spectrophotometric methods were developed andvalidated as per ICH guidelines Q2 (R1). The proposedmethods were found to be simple, rapid, precise, consistentand accurate for determination of Cycloserinein capsule dosage form. Results suggest that thesemethods can be used for routine estimation of Cycloserinein bulk and pharmaceutical dosage forms.

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