IJBPAS, June, 2021, 10(6): 2065-2071

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DEVELOPMENT AND VALIDATION OF CYCLOSERINE BY UV SPECTROPHOTOMETRIC METHOD

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Received 19th July 2020; Revised 18th Aug. 2020; Accepted 25th Sept. 2020; Available online 1st June 2021

https://doi.org/10.31032/IJBPAS/2021/10.6.5544

ABSTRACT

Analytical method validation is a process which has been demonstrated to provide a high degree of assurance that uniform batches will be produced a result that meet the required specifications. It is a process to confirm that the analytical procedure used for a specific test is suitable for intended use and help to improve identity, safety, quality, purity and potency of the drug substance.

Cycloserine is an Antibiotic used to treat tuberculosis. The physiochemical characterization studies showed that Cycloserine is white to pale yellow crystalline powder, hygroscopic in nature and has melting point is 155 to 156 °C. Cycloserine is soluble in water, slightly soluble in methanol, propylene glycol .Develop a simple, precise and accurate UV Visible method for cycloserine drug. After considering the solubility water was selected as a solvent. Cycloserine showed maximum absorbance at 226.80 nm. In this method the linearity is observed between the range of 5-25 µg/ml. Precision, Robustness, Ruggedness parameter were studied. Also Limit of Detection (LOD) and Limit of Quantitation (LOQ) were validated. The method or all parameter were validated with the help of ICH guideline.

Keywords: Cycloserine, Spectrophotometric method, Validation

INTRODUCTION

Ultraviolet spectroscopy is a technique of the spectroscopy that uses light in the visible, UV (ultra violet), and NIR (Near infrared Ranges). The Beer –Lambert Law states that the absorbance of a solution is directly proportional to the concentration of

the absorbing species present in the solution. Validation is a analytical process used to give a assurance that specific process will consistently produced and product meeting its predetermined specifications. The result of method validation can be used to determine quality, reproducibility and consistency of analytical result **[1]**.

Cycloserine is an analogue of the amino acid D-alanine. It is a broad spectrum antibiotic and has a glycinergic activities. Cycloserine is a broad spectrum antibiotic. It is a second line agent used for treatment of drug resistant tuberculosis agents caused by *Mycobacterium tuberculosis*. .D-Cycloserine is a 4-amino -1,2-oxazolidine 3-one that has R configuration. Cycloserine is an Antibiotic. It is produced by *Garyphalus* or *S. orchidaceus*. And it is used for the treatment of Tuberculosis. Its molecular formula is $C_3H_6N_2O_2$ with molecular weight 102.09 g/mol **[2]**.

Cycloserine is stable under basic conditions, with the greatest stability at pH =11.5., the half-life in patients with normal renal functions is 10 hours and is prolonged in patients with impaired renal function. The bioavailability is 70 to 90 %.

Cycloserine contains not less than 98.0 % and not more than 100.5% of $C_3H_6N_2O_2$ **[3]**.

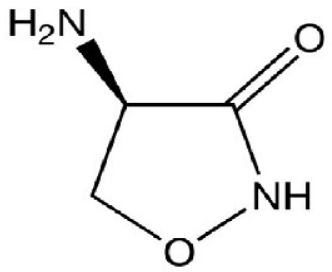

Figure 1: Chemical Structure of Cycloserine

Methods Validation:

It establishing the documented evidence ensure that a selected analytical procedure will give reproducible and reliable results that are adequate for the intended purpose.

Validation is defined as a procedure that demonstrate that a process under standard condition and ensure that provides consistent, reliable and accurate data. The parameter described in the process are according to ICH guideline. It include Linearity, Precision, LOD, LOQ, Robustness and Ruggedness.

The test method used for validation should be validated and always be verified and should be well documented **[4]**.

ICH Guidelines (ICH Q2 R1) for analytical procedure and Validation

The ICH Guideline describe the steps to perform each analytical test.

MATERIAL AND METHOD

Chemical

Cycloserine was procured a gift sample from Flamingo Pharmaceutical, Taloja, and Navi Mumbai, India. All chemicals and reagents used have a analytical reagent (AR) grade and purchased from Qualigens Fine Chemicals, Mumbai, India **[5]**.

Instruments and Apparatus

Shimadzu UV 1800 double beam spectrophotometer was used for all the spectrophotometric method. The reference and standard solutions were used for the spectra. For the scanning of solution 1 cm quartz cells is used over the range of 200 - 400 nm range. The equipment was controlled by a PC and installed properly with the UV probe software **[6]**.

Model – SHIMADZU UV 1800

Make – SHIMADZU COOPERATION ANALYTICAL INSTRUMENT

Method Development

Preparation of standard solution

The Standard stock solution was prepared with solvent water. The stock solution was prepared by accurately weighing 25 mg of Cycloserine into 25 ml of water .Then withdraw 10 ml from above solution and makeup the volume with 100ml water. The 100 ppm solution was prepare. Then it is further diluted with water to get concentration like 5 ppm **[7]**.

Selection of wavelength

The solution of 25µg/ml was scanned in the range of 400- 200 nm on spectrum mode using water as a reference.

Cycloserine shows λmax at 226.80. The wavelength selected for the analytical study was 226.80 nm **[8]**.

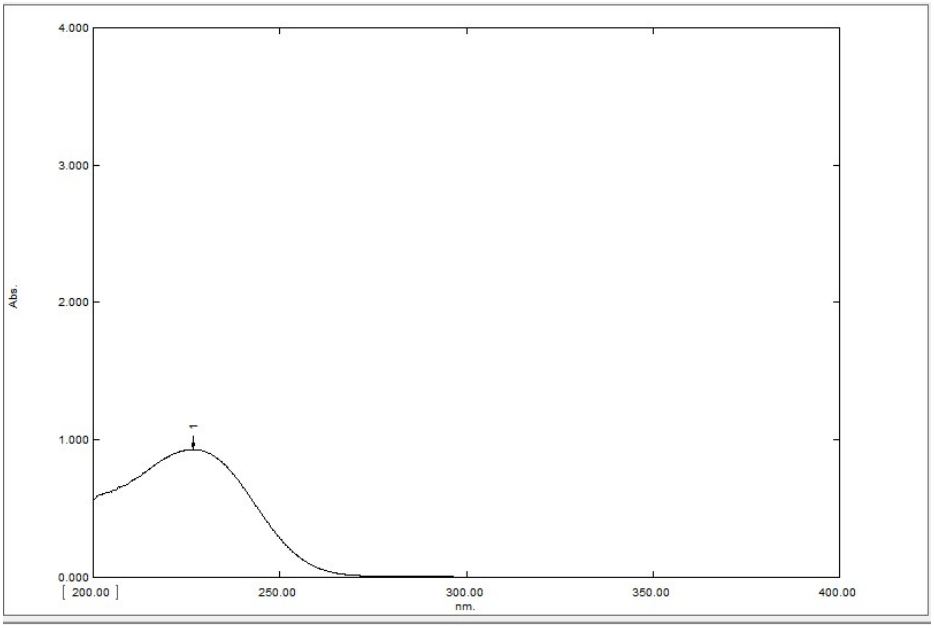

Figure 2: UV spectrum of Cycloserine

Linearity

The linearity of an analytical method is ability (within given range) to obtain test results that are directly proportional to the concentration (amount) of the analyte in samples within given range. Standard solution will be prepared at five concentrations. Further calibration curve of cycloserine was plotted by measuring absorbance of 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml solutions at a λmax 226.80 nm. Spectrophotometric data for the estimation of Cycloserine at 226.80nm. In linearity the regression equation was calculated **[9] (Figure 3)**.

Limit Of Detection and Limit Of Quantitation:

The LOD and LOQ of cycloserine by validation method were determined by using calibration graphs. LOD and LOQ were calculated as

LOD = 3.3 X SD /Slope

LOQ = 10 X SD /Slope

Where SD is the Standard Deviation.

Precision

In intraday study the concentrations of replicates of drug was prepared and calculated on the same day for three times. Calculate the mean assay, % Deviation and % Relative standard deviation **[10] (Table 3)**.

Robustness

Robustness measures the capacity of an analytical method to remain unaffected by small but deliberate variations in method parameters. Robustness provides some indication of the reliability of an analytical method during normal usage.

Robustness is usually performed by making small deliberate changes in the operating parameter and comparing the results to those obtained using the prescribed method **[10] (Table 4)**.

Ruggedness

The ruggedness of the method was determined by carrying out the analysis by the different analyst. The different analyst taken a reading at same concentration. And calculate the % RSD **[10] (Table 5)**.

Table 1: Spectrophotometric data for the estimation of Cycloserine at 226.80 nm

Sr. No.	Concentration (µg/ml)	Absorbance
1	0.00	0.00
2	5.00	0.296
3	10.00	0.48
4	15.00	0.621
5	20.00	0.803
6	25.00	0.999

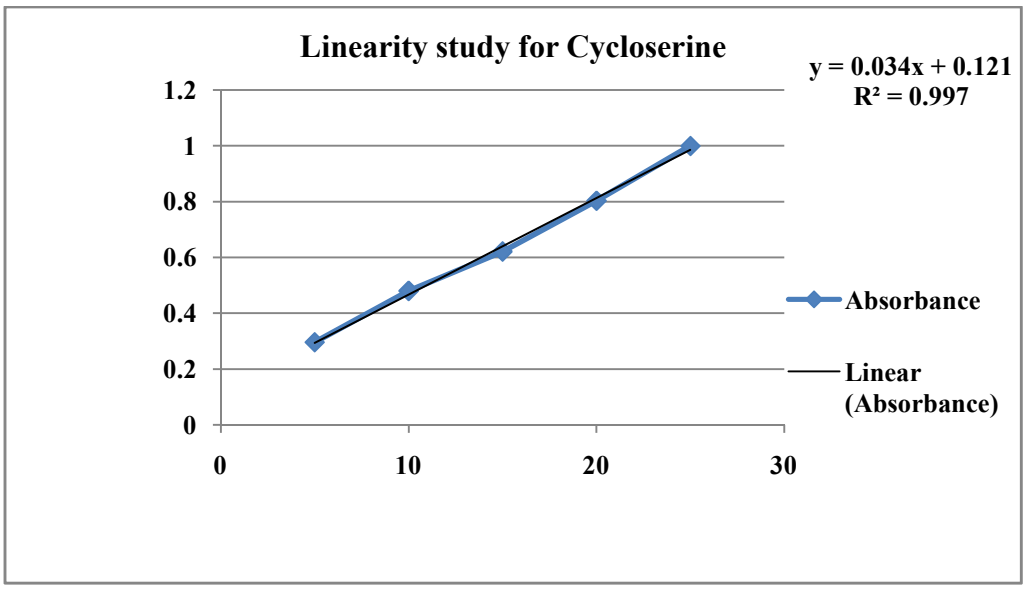

Figure 3: Calibration curve of Cycloserine

Table 2: Linearity study of cycloserine

Sr. No.	Concentration (µg/ml)	Absorbance	Regression Data		
1	5	0.296	m* = 0.0346	c* = 0.1211	R^2 = 0.9973
2	10	0.48			
3	15	0.621			
4	20	0.803			
5	25	0.999			

m*= slope ; c=intercept; r =regression

Table 3: Intra Day Precision Study

Intraday Precision for Cycloserine				
Sr. No.	Conc.(ppm)	Abs. I	Abs. II	Abs. III
1	15	0.667	0.669	0.651
2	15	0.662	0.660	0.612
3	15	0.669	0.671	0.655
4	15	0.665	0.623	0.688
5	15	0.661	0.645	0.633
6	15	0.662	0.629	0.699
Average		0.6643	0.6495	0.656333
SD		0.003204	0.020472	0.032752
%RSD		0.482313	3.151953	4.990085
Average %RSD		2.8747		

Table 4: Robustness Study for Cycloserine

Sr. No.	Conc.(ppm)	225nm	226.80nm	227nm
1	25	0.983	0.988	0.99
2	25	0.984	0.988	0.99
3	25	0.984	0.987	0.989
4	25	0.982	0.985	0.99
5	25	0.985	0.986	0.989
6	25	0.985	0.989	0.989
Mean		0.983833	0.987167	0.9895
SD		0.001169	0.001472	0.000548
RSD		0.118825	0.14911	0.055353
%RSD		11.88	14.91	5.535
Average %RSD		10.775		

Table 5: Ruggedness study for Cycloserine

Sr. No.	Conc.(ppm)	Analyst 1	Analyst 2	Analyst 3
1	20	0.830	0.831	0.830
2	20	0.827	0.825	0.826
3	20	0.820	0.821	0.820
Mean		0.82566	0.82566	0.82533
SD		0.00513	0.00503	0.00503
RSD		0.00621	0.00609	0.00609
%RSD		0.62151	0.60959	0.60984
Average %RSD		1.10992		

RESULT AND DISCUSSION

The λmax of cycloserine was 226.80 nm. The slope (m) of calibration curve of cycloserine was 0.0346.the linearity studies showed that estimation of cycloserine between 5 µg/ml -25 µg/ml was found to be linear with slope (m) -0.0346 ,intercept (c) is 0.1211 (r^2 = 0.9973) . The solubility of cycloserine follows the order water>methanol>propylene glycol. The physiochemical characterization studies of cycloserine showed that the active sample obtained was pure .The analytical work for the estimation of cycloserine was found to be obey Beer's law and the curve was found to be linear.

Parameter	Result
Linearity Range	5-25 µg /ml
Correlation Coefficient	0.9973
Precision indicated by %RSD	2.8747%
Limit of Detection (LOD), µg /ml	26.10
Limit of Quantitation (LOQ), µg /ml	79.10
Linear Regression Equation	Y=0.0346x+0.1211
Robustness indicated by %RSD	10.775
Ruggedness indicated by %RSD	1.1092

CONCLUSION

The proposed method is simple, precise and less time consuming method has been developed for the quantitative estimation of cycloserine. This method is developed as per ICH Guideline. The developed method is successfully used for the quantification of cycloserine drug substances in routine analysis.

Acknowledgment

We are thankful to our guide Prof. Patel S. G. for providing all the facilities and guidance to carry out the research .We would like to thank Flamingo Pharmaceutical, Taloja, Navi Mumbai, India for providing the drug Cycloserine.

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