IJPSR (2023), Volume 14, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 October 2022; received in revised form, 05 December 2022; accepted 30 April 2023; published 01 June 2023

EVULSIONS SPECTROPHOTOMETRIC DETERMINATION OF CYCLOSERINE IN PHARMACEUTICAL FORMULATIONS WITH BPB AND BCG DYES

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Keywords:

Spectrophotometry, Cycloserine, Bromo phenol blue, Bromo cresol green, Ion – pair complex, ICH Validation

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ABSTRACT: Two simple and sensitive evulsions spectrophotometric methods have been described for the assay of Cycloserine either in pure form or in pharmaceutical formulations. The developed methods involve formation of coloured chloroform extractable ion - pair complexes of the drug with bromophenol blue (BPB) and bromo cresol green (BCG) in acidic medium. For two methods, the extracted complexes showed absorbance maxima at 408 and 419.5nm. Beer's law is obeyed in the concentration ranges 2.0-20 and 2.5-25μg/mL with BPB and BCG respectively. The effects of concentration of dye, pH and interference of recipients have been studied and optimized. The limit of detection and quantification has been determined for two methods. These two are very accurate methods of physical and instrumental mode of experiments. Two methods have been validated as per the guidelines of ICH. The methods have been applied to the determination of drug in commercial tablets and results of analysis were validated statistically through recovery studies.

INTRODUCTION: Cycloserine **Fig. 1** is chemically known as 4-amino-1, 2-oxazolidin-3-one with R configuration. It is an antibiotic produced by Streptomycin garyphalus or S - Orchidaceous and is used as part of a multi-drug regimen for the treatment of tuberculosis when resistance to, or toxicity from, primary drugs has developed. Cycloserine, sold under the brand name *Seromycin* and *Cyserin*, is a GABA transaminase inhibitor and an antibiotic, used to treat tuberculosis [1, 2]. Specifically it is used, along with other antituberculosis medications, for active drug resistant tuberculosis.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(6).3005-11

This article can be accessed online on www.ijpsr.com

DOI link: http://doi.org/10.13040/IJPSR.0975-8232.14(6).3005-11

The literature survey revealed only few methods is available. The determination of Cycloserine in dosage forms and include Cycloserine is an antibiotic used to treat and prevent a number of bacterial infections. These include pneumonia, urinary tract infections and Lyme disease [3, 4]. It is used by injection into a vein or muscle [5, 6].

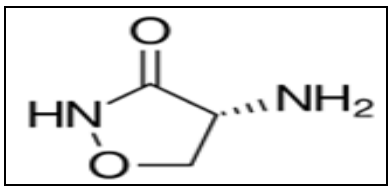

FIG. 1: CYCLOSERINE

Broad spectrum [7] cephalosporin antibiotic resistant to beta - lactamase. It has gram-negative and gram-positive organisms, and haemophilias. Semi synthetic cephalosporin antibiotics are administered for treating various bacterial infections such as pharyngitis, chronic bronchitis and uncomplicated

gonorrhoea and septicaemia [8]. A second-generation cephalosporin is administered orally and in the parenteral dosage form [9]. The mode of action of Cycloserine against the bacteria is to inhibit bacterial wall synthesis by binding proteins. Some of the analytical methods reported for the assay of Cycloserine in the marketed form include spectrophotometry [10], High-performance liquid chromatography (HPLC) [11, 12], Capillary electrophoresis [13, 14, 15], HPTLC [15] and LC-MS/MS [16]. The current communication proposes a simple analytical procedure to quantify Cycloserine in pure and trade products using HPLC [11, 12]. The proposed HPLC is based on the separation of Cycloserine using ion pair reagents. The methods for the determination of Cycloserine in human plasma described in the literature used an HPLC with UV detection [18, 19, 20] and a liquid chromatography coupled to mass spectrometry [17]. The LC-MS/MS methods were substantially more sensitive and allowed for Cycloserine quantification in the 25-50 µg/mL range using only 100 μL.

MATERIAL AND METHODS: Cycloserine was procured from Srini Pharmaceuticals Limited, Hyderabad as a gift sample. The dyestuffs *viz.*, BPB and BCG (AR grade) supplied by SD Fine Chemicals Ltd. Mumbai, were used without any further purification. The dyestuffs were used as 0.025% solutions in doubly distilled water. Sodium acetate - hydrochloric acid buffers of pH 2.8, and 3.5 were prepared by mixing 50mL of 1M sodium acetate solution with 49.50 and 46.25 mL respectively, of 1M HCl solution and diluted to 250 mL with doubly distilled water. The pH of each solution was adjusted to an appropriate value with the aid of a pH meter. Chloroform (HPLC grade) supplied by SD Fine Chemicals Ltd. Mumbai was used throughout the experiment. Stock solutions were prepared for two dyes and drugs (25mg/100mL). The spectra 2 and 3 **Fig. 2, 3** of ion-pair complexes have been recorded on Elico double beam SL 210 spectrophotometer using quartz cells of 10mm path length. An Elico model Li -120 pH meter was used for pH.

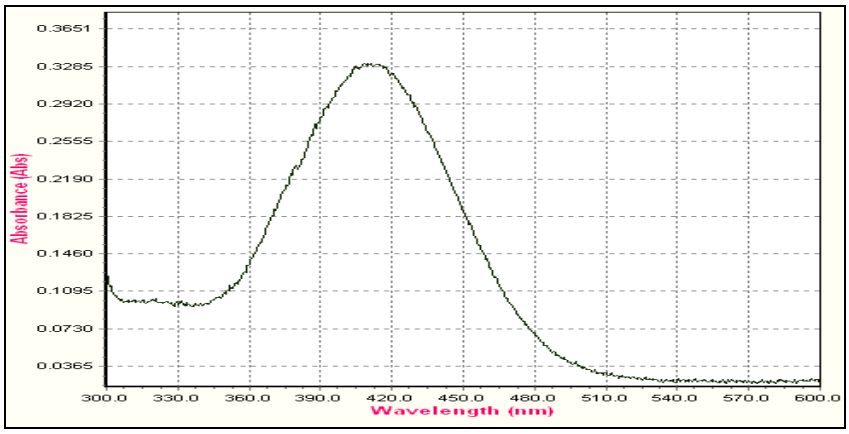

FIG. 2: ABSORPTION SPECTRUM OF CYCLOSERINE. Bromo phenol blue Complex extracted into 10 mL chloroform [drug] = 25 mg mL^{-1} + 5 mL of 0.025% BPB + 5 mL of *p*H 2. 8 buffer

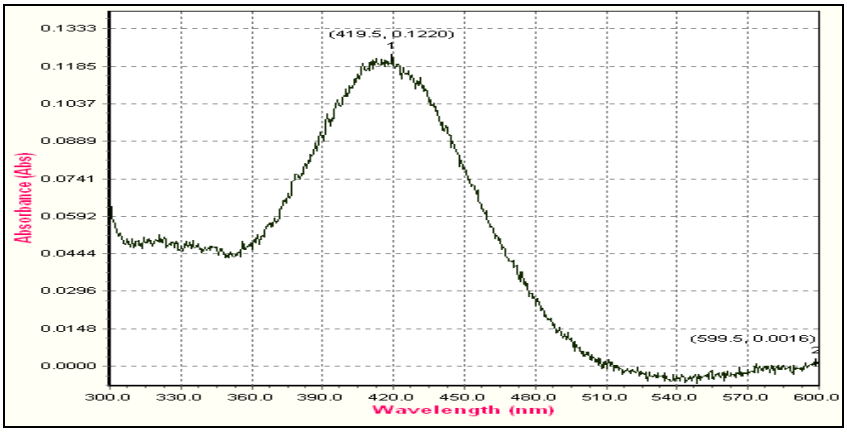

FIG. 3: ABSORPTION SPECTRUM OF CYCLOSERINE. Bromo cresol green Complex extracted into 10 mL chloroform [drug] = 25 mg mL^{-1} + 5 mL of 0.025% BCG + 5 mL of *p*H 3.5 buffer

Calibration Curve: Different aliquots of drug solution were transferred into 125 mL separating funnel. To this 5 mL of buffer (pH 2.8 and 3.5), 5 mL of dye was added, and the total volume was made up to 20 mL with water. 10 mL of chloroform was added and the contents were shaken for 5 minutes. The two layers were allowed to separate for 5 minutes. The organic layer was separated and the absorbance of yellow colour solution which is stable at least for 3 hours is measured at 420nm against blank similarly prepared. The same procedure of analysis is followed either for assay of pure drug or for dosage form. The calibration graphs **Fig. 4** are linear over the concentration ranges and within the permissible range. The optical characteristics and statistical data for the regression equation of the proposed methods are presented in **Table 1.**

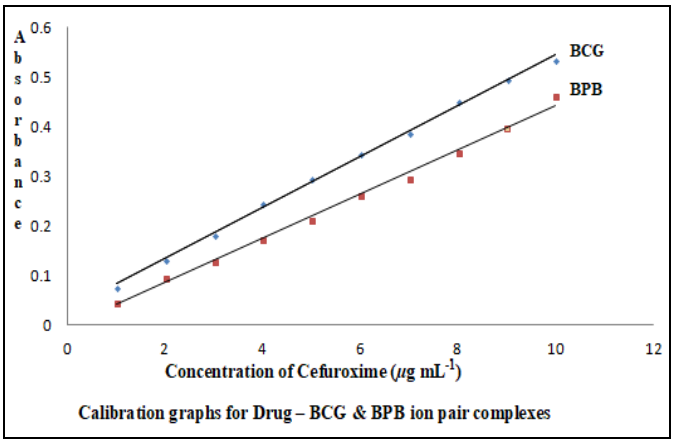

FIG. 4: CALIBRATION GRAPH

TABLE 1: OPTICAL CHARACTERISTICS AND STATISTICAL ANALYSIS FOR THE REGRESSION EQUATION OF THE PROPOSED METHODS FOR THE ESTIMATION OF CYCLOSERINE

	Extraction methods with	
Parameters	**BCG**	**BPB**
	λ_{max} (nm)	
Beer's law limit (μg mL^{-1})	2.5 – 25	2.0 – 20
Molar absorptivity (L mol^{-1} cm^{-1})	26121	20732
Formation constant K, M^{-1}	2×10^{6}	1.92×10^{6}
Sandell sensitivity (μg cm^{-2})	0.0204	0.0227
Slope (Specific absorptivity), b	0.0514	0.0446
Intercept (a)	0.03106	-0.00426
Correlation coefficient (r)	0.999	0.997
Standard deviation of intercept (% n = 6)	0.10152	0.07887
Limit of detection, (μg mL^{-1})	6.51782	5.83567
Limit of quantification (μg mL^{-1})	19.7509	17.6838
Regression equation[a]	Y= 0.0514C+0.03106	Y=0.0446C+(-0.00426)

[a] with respect to Y = b c + a, Where C is the concentration (μg mL^{-1}) and Y is absorbance [b] six replicate samples.

Procedure for the Assessment of Pure Drug: Five different solutions of pure drug in the range of calibration curve were selected and the recovery investigations were performed. The reclaims and their relative standard deviations are tabulated in **Table 2.**

TABLE 2: APPLICATION OF PROPOSED METHODS FOR THE ANALYSIS OF CYCLOSERINE IN PURE IN FORM

Proposed methods Taken (μg mL^{-1})	**Reference method**				
	Found (µg mL-1)		**Recovery (%)**		**Recovery (%)**
	BCG	**BPB**	**BCG**	**BPB**	
2.5	2.54	2.52	101.6	100.8	98.25
5.0	5.11	4.98	102.2	99.60	101.25
7.5	7.58	7.65	101.06	102	101.65
10.0	9.98	9.89	99.8	98.9	101.16
12.5	12.46	12.60	99.68	100.8	100.60
					99.00
					103.58
					101.95
RSD (%)			0.1006	0.0785	1.0615
Mean±SD			101.20 ± 1.02	100.90± 0.89	101.08 ± 1.63
t – test			0.1421	0.5664	
F – test			0.8512	0.5140	

Procedure for the Assessment of Dosage forms: Ten capsules of CYSERIN – 250mg Cipla Company and powdered and dissolved in doubly distilled water, stirred thoroughly, and filtered through a Whatman No. 42 filter paper. This solution was transferred into 100 mL standard flask and diluted with doubly distilled water as required. Different solutions of drug in the range of calibration curve were chosen and the assessment was estimated using the calibration curve. The results of the recovery investigations are tabulated in **Table 3.**

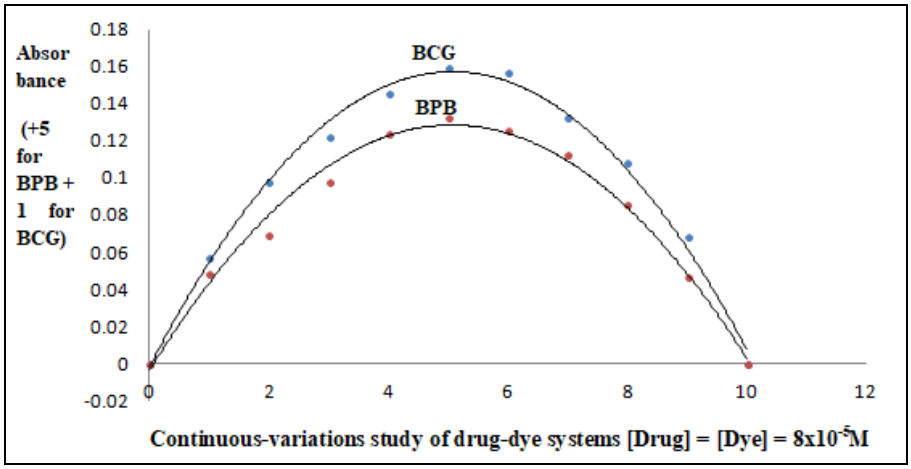

FIG. 5: CONTINUOUS VARIATION GRAPH

TABLE 3: APPLICATION OF PROPOSED METHODS FOR THE STUDY OF CYCLOSERINE IN PHARMACEUTICAL FORM

Proposed methods Taken ($\mu g\ mL^{-1}$)	Reference method				
	Found (µg mL-1)		Recovery (%)		Recovery (%)
	BCG	BPB	BCG	BPB	
3	3.06	2.98	102	99.33	98.25
6	6.11	6.03	101.83	100.50	101.25
9	9.08	9.04	100.88	100.44	101.65
12	11.91	12.13	99.25	101.08	101.16
15	15.13	14.82	100.86	98.80	100.60
					99.00
					103.58
					101.95
RSD (%)			0.1005	0.0780	1.0615
Mean±SD			100.964±1.02	100. 03±0.89	100.497 ±1.63
t – test			0.1421	0.5664	
F – test			0.8512	0.5140	

RESULTS AND DISCUSSION: Cycloserine forms ion-pair complexes in acidic buffer with dyestuffs such as bromophenol blue (BPB) and bromocresol green (BCG), and these complexes are extracted into chloroform.

Ion-pair complexes of drug with BPB and BCG absorbed maximally at 408 and 419.5 respectively. The reagent blank under similar conditions showed no absorption.

Cycloserine molecule has one amide nitrogen and one amine -NH_2 group. The protonation takes place preferably on free amino nitrogen since the approach of dye molecule to drug molecule from amine -NH_2 group. The sulphonic acid group present in BPB and BCG undergoes dissociation in the *p*H range 1 to 5. The colour of such dyes is due to the opening of lactoid ring and subsequent formation of quinoid group.

It is supposed that the two tautomers are present in equilibrium but due to strong acidic nature of the sulphonic acid group, the quinoid body must predominate. Finally the protonated Cycloserine forms ion-pairs with the dyestuffs which are quantitatively extracted into chloroform. The possible structure of ion-pair complex is proposed and given in Chart -1 and 2

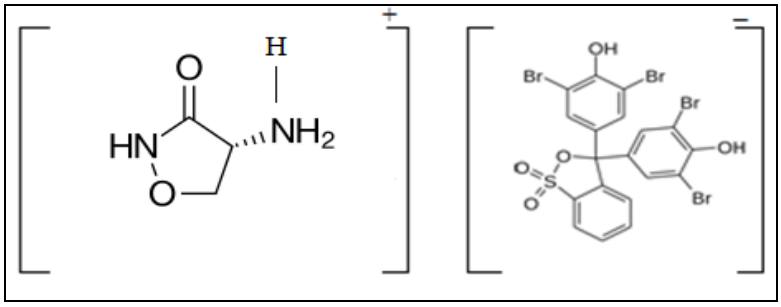

CHART 1: CYCLOSERINE – BPB ION - PAIR COMPLEX

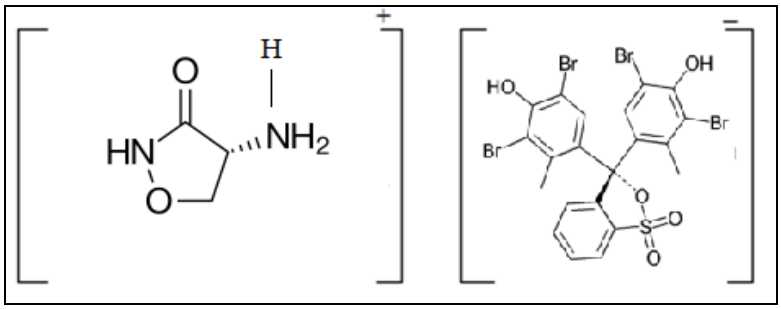

CHART 2: CYCLOSERINE – BCG ION - PAIR COMPLEX

The effect of pH on the ion-pair formation of Cycloserine with various dyestuffs has been studied using sodium acetate hydrochloric acid buffer. The results are shown in **Fig. 6**. It is observable that absorbance of complexes with BPB and BCG was found to be constant within the pH ranges 2.0-3.0 and 2.8-3.8, respectively. Thus, all the absorbance measurements were formed at pH 2.5 and 3.5 with BPB and BCG, respectively.

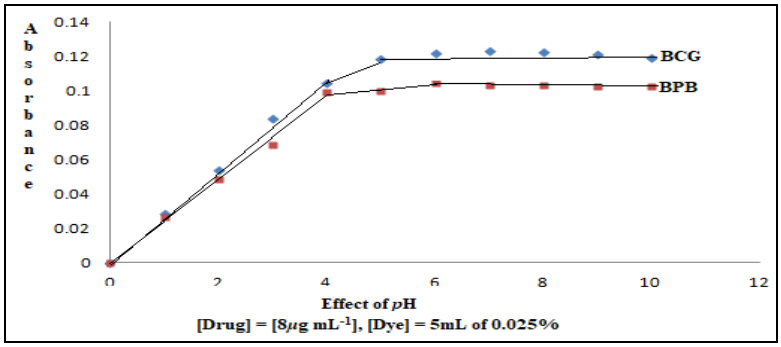

FIG. 6: EFFECT OF *pH* ON ABSORBANCE OF BPB & BCG COMPLEXES

The influence of dyestuff concentrations was also studied by adding unlike volumes of dyestuff to a constant amount of Cycloserine (8 μg mL^{-1}). It is apparent from **Fig. 7** that the maximum absorbance, in each case, was found with 3.0 mL of dyestuff, beyond which absorbance was constant. Thus, 5 mL of each dyestuff was used for ion-pair formation [21, 22, 23] throughout the experiment.

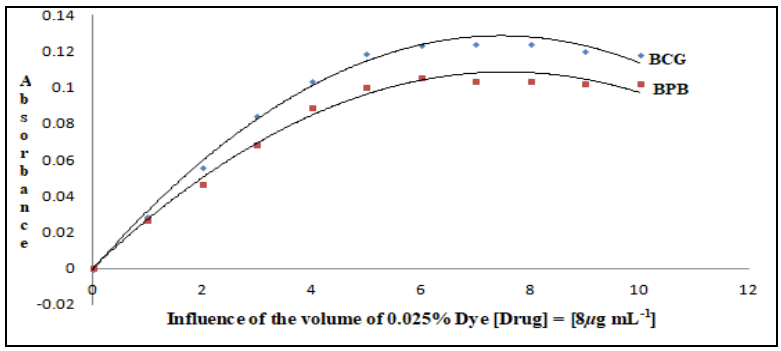

FIG. 7: IFLUENCE OF VOLUME ON ABSORBANCE OF BPB & BCG COMPLEXES

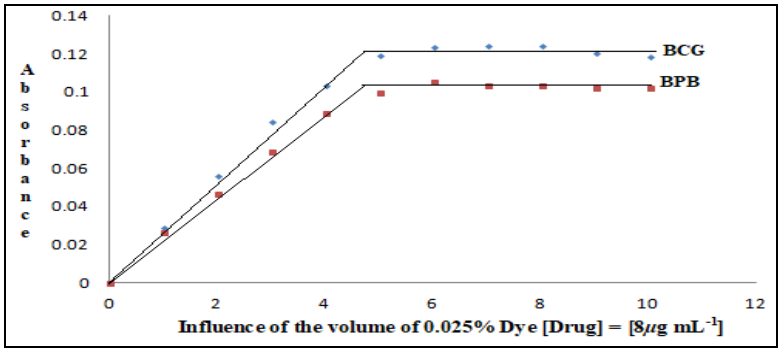

FIG. 8:

A systematized study of the effect of foreign species present along with Cycloserine on the determination of Cycloserine at 8 μg mL^{-1} levels was accepted. This study followed the proposed procedures for a 10 mL sample system, adding a known amount of foreign species to a Cycloserine solution of 8 μg mL^{-1}.

Table 4 summarizes the results obtained. However, the drug content from the powdered capsules was extracted into chloroform, which completely removes any interference by the common excipients found in formulations.

TABLE 4: INTERFERENCE STUDY

S. no.	Excipients	Tolerance limit (μg mL^{-1})
1	Microcrystalline cellulose	89
2	Starch	178
3	Lactose	126
4	Povidone	64
5	Silicon dioxide	92
6	Titanium dioxide	49

Validation of the Proposed Method: The two suggested methods have been validated in terms of guideline proposed by the International Conference on Harmonization [24] *viz.*, selectivity, specificity, accuracy, precision, limits of calibration curve, LOD, LOQ, robustness, ruggedness and regression equation. The student *t*-test and variance F-test have been performed compared to a reference method.

The suggested methods have been successfully applied to determine Cycloserine in pharmaceutical preparations. The performance order of the suggested methods is BCG > BPB. And compared to those obtained by a reference method (ICH, 1996) by means of a *t*-test at 95% confidence level. In all cases, the average results obtained by the suggested and reference methods were statistically identical, as the difference between the mean values had no significance at 96% confidence level. The proposed methods are simple, sensitive, and reproducible and can be used for regular analysis of Cycloserine in pure form and formulations.

CONCLUSION: In conclusion, Cycloserine forms ion-pair complexes with acidic triphenylmethane dyes viz., bromophenol blue and bromocresol green in 1:1 proportion. These complexes are evulsions into chloroform and offer a basis for the assay of the drug. The developed methods are simple, sensitive, and reproducible and can be used for routine analysis of Cycloserine in pure and formulation forms.

ACKNOWLEDGEMENTS: The authors are grateful to Dr. T. Veeraiah Principal (Retd.), S. A. P. College, Vikarabad, for helpful discussion and Dr. M. Jangareddy, Director, CMR Institute of Technology, Hyderabad.

CONFLICTS OF INTEREST: There is no conflict of interest.

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How to cite this article:
Ravi M, Ramya E and Padmavati K: Evulsions spectrophotometric determination of cycloserine in pharmaceutical formulations with BPB and BCG dyes. Int J Pharm Sci & Res 2023; 14(6): 3005-11. doi: 10.13040/IJPSR.0975-8232.14(6).3005-11.