DEVELOPMENT AND VALIDATION OF EXTRACTIVE SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF CHLORDIAZEPOXIDE IN TABLET DOSAGE FORMS

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Abstract:

Two visible spectrophotometric methods (Method A and B) were developed and validated for the determination of Chlordiazepoxide in bulk and tablet dosage forms. In these spectrophotometric methods, the chromogenic reagents such as Alizarin Red S dye (Method – A) and Aconitic Acid (Method – B) reagents were used for color development for the estimation of Chlordiazepoxide in pure and formulations. The developed methods were validated according ICH guidelines and adopted for the assay of chlordiazepoxide in the bulk drug and formulations.

Key words: Chlordizepoxide, Alizarin Red S dye, Aconitic Acid, Validation, Assay

Introduction:

Chlordiazepoxide (initially called methaminodiazepoxide) was the first benzodiazepine (Fig. 1) used to treat anxiety and acute alcohol withdrawal. It is also used to relieve fear and anxiety before surgery.[1,2]
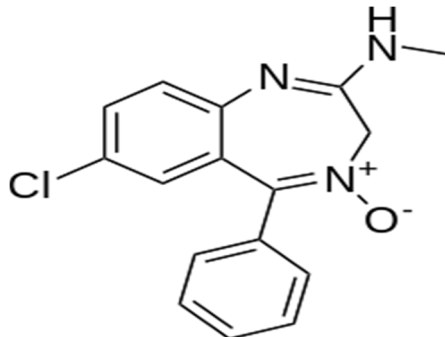

Figure 1: Structure of Chlordiazepoxide

The drug has amnestic, anxiolytic, hypnotic and skeletal muscle relaxant properties [3].

Chlordiazepoxide acts on the components of Central Nervous System like brain and spinal cord to produce calming effect by enhancing the effects of GABA. It is used in conditions like acute intoxication with narcotics, severe liver deficiencies, severe sleep apnea and when allergic to other benzodiazepine drugs.[4]

Drowsiness and confusion are the common side effects. But, mood changes, trouble walking, trouble urinating and sleep disturbances are also reported.[5] Very few methods of quantitative analysis are so far reported like HPLC[6,7], HPTLC[8] and spectrophotometry[9,10] which are all simultaneous methods.

Materials and Methods:

Chlordiazepoxide reference standard was provided by East West Pharma Pvt. Limited, Hyderabad, formulation tablets were purchased from a local pharmacy (Med Plus). All chemicals and reagents such as Alizarin Red S, Citric Acid, Acetic Anhydride, Chloroform and Methanol were of AR grade and were purchased from Merck Specialities Pvt. Ltd., Mumbai.

Instrumentation: In Method A and B, a UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Denver electronic analytical balance (SI234) and Systronics digital pH meter were used for weighing and to adjust pH of the mobile phase.

Preparation of solutions: An amount of the pure drug equivalent to 10mg of Chlordiazepoxide was accurately weighed and transferred into a 10ml volumetric flask, dissolved with 10ml of MeOH, sonicated for five minutes and filtered through Ultipor N66 Nylon 6, 6 membrane sample filter paper. The filtrate is made up to the mark with the same solvent. To prepare sample solution, the average weight of five tablets of Chlordiazepoxide (Ebrium -10 mg) was determined and powdered them with mortar. An amount of powder equivalent to 10mg of Chlordiazepoxide was weighed accurately and transferred into a clean 10 ml volumetric flask and dissolved in methanol. Then the solution was made up to the volume with same solvent. The solution was sonicated for 5min and filtered through 0.45 µm membrane filter. Then appropriate volumes of the solution were further diluted with the solvent to prepare solutions of required concentration.

Preparation of reagents: 0.2% ARS solution was prepared by dissolving 200mg of Alizarin Red S in double distilled water and made up to 100ml. Citric Acid – Anhydride solution is prepared by dissolving 1.2g of Citric acid in 5.0mL of Methanol and made up to 100mL with acetic anhydride. About 8.7ml of concentrated HCl was transferred

into a clean 100ml volumetric flask and diluted up to the mark with distillated water and the final concentration of the resulting solution was 1N HCl.

Method Development:

In developing visible spectrophotometric methods, optimized conditions were developed by making different trails by varying one of the parameters such as concentration of standard, volume of the reagents, order of addition of the reagents, temperature and time for color development and keeping other as constant, and the optimized procedures were presented below.

Method - A: About 2.5ml of stock solution (200 µg/ml) was accurately transferred into 125 ml separating funnel, about 6.0 ml of HCl solution and 2.0 ml of 0.2% dye solution were added. The volume of aqueous phase is adjusted to 15 ml with distilled water. Then 10mL of chloroform is added and the contents are shaken thoroughly for 2 min. and the two phases are allowed to separate completely. Absorbance of the colored chloroform layer was scanned over a range of wavelength 400-800nm against a reagent blank and the wavelength of maximum absorbance was found to be 498nm. The absorption spectrum was presented in Figure-2.This method was based on the formation of ion associated colour complex between the dye and the drug.
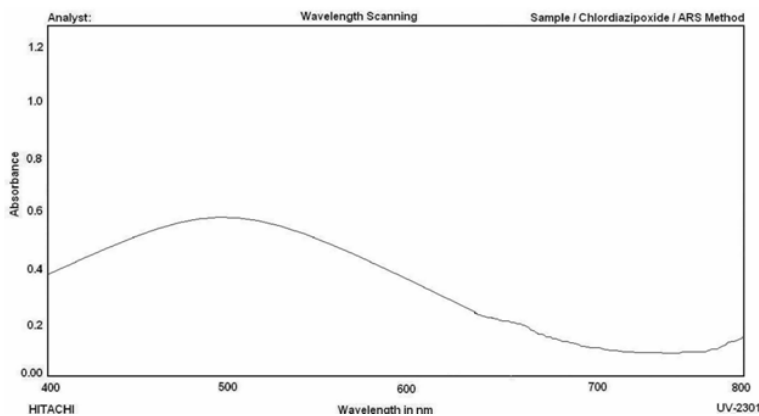

Figure 2: Wavelength Scanning spectrum: Method – A

Method - B: In this method, in a 25ml graduated tube aliquot of standard drug (100 µg/ml) is gently evaporated to dryness on a boiling water bath. 10 mL of Citric acid – acetic anhydride reagent is added and the flask is kept immersed in boiling water in the bath. After 30 min. the flask is cooled to room temperature and the contents are diluted to the mark with anhydride. Acetic anhydride dehydrates citric acid to form aconitic acid that forms violet chromogen by becoming internal salt with amine group of the drug molecule. The absorbance of the chromogen was scanned against a reagent blank and the absorbance maxima was found to be 587nm and the spectrum is presented in Figure – 3.
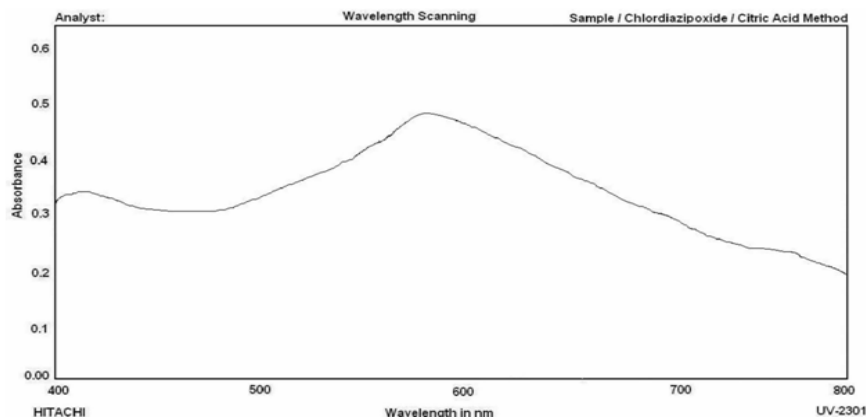
Figure 3: Wavelength Scanning Spectrum: Method – B

Method Validation Specificity: Absorbance was measured for the colored products of the standard and sample in Method-A and Method-B. The experimental results were given in Table-1.

Precision: The precision of the proposed methods was expressed in terms of intra-day and inter-day precision. The intraday and inter day precision were determined by measuring the response of the instrument (absorbance) six times within two different days by freshly preparing working standard solution and measuring the signal of the instrument following the standard procedures for the Method A and B. The precision was expressed as percent of standard deviation (%RSD). The results of intraday and inter day precision results were found to be 0.65 & 0.36 for Method – A and 0.31 & 0.0.37 for Method – B respectively.

Accuracy: Accuracy of the proposed methods was determined by calculating percent of recovery of Chlordiazepoxide by the method of standard addition and the results are presented in Table-2.

Linearity: In Method-A, different aliquots of stock solution were accurately transferred into a series of 125 mL separating funnels and the chromogen is formed by adopting the optimum procedure stated above. The absorbance of the colored solutions obtained are measured at 498nm against a reagent blank and a linear plot was drawn for absorbance against concentration and represented in Figure 4.
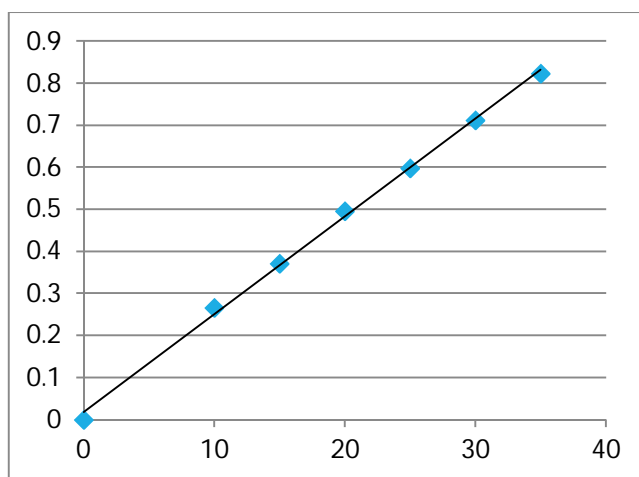

Figure 4: Linearity graph – Method A

In case of Method-B, the absorbance of the chromogen solutions formed was measured at 587nm against a reagent blank and calibration plot was constructed and presented in Figure-5. The results of linearity in these methods were presented in Table-3.
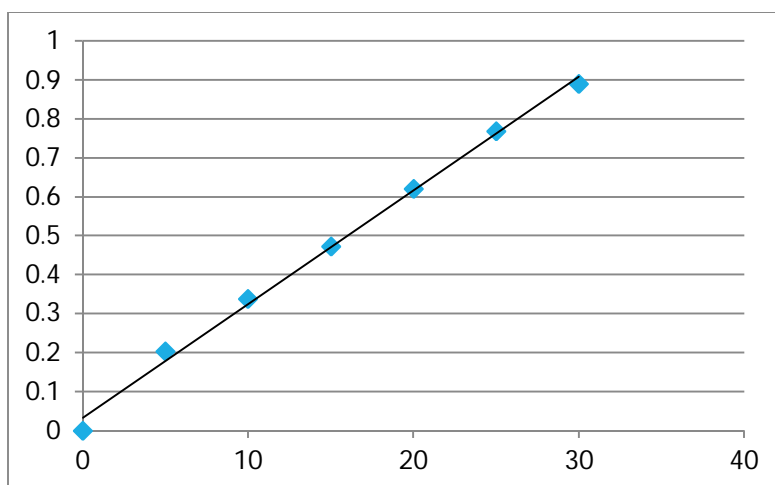

Figure 5: Linearity graph – Method B

Sensitivity: The sensitivity of the proposed method was presented in terms of limit of detection (L.O.D.) and limit of quantification (L.O.Q.) and these were calculated from standard deviation of response and slope of the calibration curve(s) by using the formulae LOD = 3Sa / b and LOQ = 10Sa / b respectively and given in Table-4.

Formulation analysis: The prepared sample solution was analyzed by adopting the standard procedures and percent of assay was determined, and the assay results were given in Table-5.

Ruggedness: The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times, different assay temperatures, different days, etc. In the present investigation, robustness was tested by performing the analysis following the recommended procedures by two different analysts and % RSD of the two proposed methods were given in Table-6. It was found that there is no remarkable change in the results with the changed condition.

Stability: The stability period of the formed color complex in the developed methods was determined my calculating the % assay of the developed color complex at different intervals in their corresponding wavelengths. It was found that the colour complex in Method-A and B are stable for 105 and 80 min. respectively.

Results and Discussion:

The main objective of the present investigation was to develop and validate two visible spectrophotometric methods using Alizarin Red S and Aconitic acid as complexing agents for color development for the determination of Chlordiazepoxide in pure and formulations.

The %RSD in intraday and inter day precision studies was found to be less than 2.0% indicate that the proposed methods were precise. The accuracy of the methods was determined as percent of recovery, and performed at three different concentrations and found to be in the range of 100.55-101.79%, this indicate that the developed methods were accurate.

The studies of linearity between the response and concentration of the drug indicate that these methods were linear within the best suitable rage (10-35 and 5-30 µg/ml for Method-A and B respectively) and correlation coefficient was found to be not less than 0.9980. The LOD and LOQ values of the proposed methods were (0.75 & 2.5 and 0.3

& 1.2) indicating that the methods are sensitive. The study of ruggedness was carried out and results proved the developed Methods are rugged. Assay of formulations was determined and the results were satisfactory, hence these methods may adopt in any quality control laboratory.

CONCLUSIONS:

The proposed methods were found to be simple, sensitive, precise, accurate, and linear over a suitable range and rugged. The tablet dosage forms were analyzed successfully and the assay was found to be within the limits, hence the proposed methods may be applied for the quality analysis.

Table-1: Specificity of the proposed methods:

	Absorbance	
	Method – A	Method – B
Standard	0.496	0.473
λ max	498 nm	587 nm

Table-2: Accuracy results for the proposed methods:

Method	Recovery	Concentration In µg/ml	Amount found in µg/ml	% of recovery*	Average Recovery
Method A	50%	15	15.24	101.79	101.18
	100%	20	20.24	101.21	
	150%	25	25.13	100.55	
Method B	50%	15	15.08	100.56	100.70
	100%	20	20.14	100.75	
	150%	25	25.2	100.82	

* Average of three determinations

Table 3: Linearity Results:

Sl.No.	Method – A		Method – B	
	Concentration in µg/ml	Absorbance	Concentration in µg/ml	Absorbance
1.	10	0.267	5	0.203
2.	15	0.372	10	0.339
3.	20	0.496	15	0.473
4.	25	0.598	20	0.621
5.	30	0.712	25	0.769
6.	35	0.824	30	0.891
Slope:	**0.023**		**0.029**	
Intercept:	**0.018**		**0.032**	
Correlation Coefficient:	**0.999**		**0.998**	

Table-4: Sensitivity of the proposed method expressed as LOD and LOQ:

Method	Method – A	Method – B
LOD [µg/ml]	0.75	0.3
LOQ [µg/ml]	2.5	1.2

Table-5: Formulation Analysis results of Chlordiazepoxide:

S. No.	Method	Formulation	Amount prepared	Amount found	% Assay
1	Method – A	Ebrium -10mg	20µg/ml	19.89	99.4
2	Method – B	Ebrium -10mg	15µg/ml	14.82	98.8

Table-6: Ruggedness Results:

Method	A	B
S. No	Absorbance at 20 µg/ml	Absorbance at 15µg/ml
1	0.534	0.448
2	0.531	0.446
3	0.533	0.444
4	0.53	0.443
5	0.532	0.447
6	0.529	0.445
%RSD	**0.35**	**0.38**

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