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Development and validation of a RP–HPLC method for the determination of chlordiazepoxide in formulations

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ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Chlordiazepoxide in tablet dosage form. Isocratic elution at a flow rate of 1mL/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) column at ambient temperature. The mobile phase consisted of Methanol: Acetonitrile: 0.1%OPA in 50:40:10 v/v, (pH 4.7). The UV detection wavelength was 217 nm and 20µL sample was injected. The retention time for Chlordiazepoxide was 3.58 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Chlordiazepoxide in tablet dosage form and bulk drug.

Key words: Chlordiazepoxide, RP-HPLC, UV detection, recovery, 217nm

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INTRODUCTION

Chlordiazepoxide (initially called methaminodiazepoxide) was the first benzodiazepine to be synthesized in the mid-1950s [1-3]. Chlordiazepoxide is used to treat anxiety and acute alcohol withdrawal. It is also used to relieve fear and anxiety before surgery. The discovery of chlordiazepoxide was by pure chance[4]. Chlordiazepoxide and other benzodiazepines were initially accepted with widespread public approval but were followed with widespread public disapproval and recommendations for more restrictive medical guidelines for its use [5]. The drug has amnestic, anxiolytic, hypnotic and skeletal muscle relaxant properties [6].

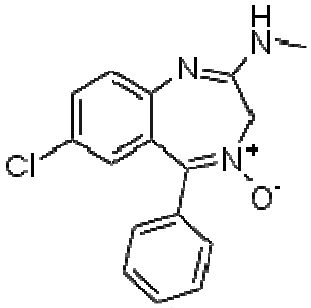

Figure 1: Structure of Chlordiazepoxide

Systematic (IUPAC) name	:	7-chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine-4-oxide
Formula	:	$C_{16}H_{14}ClN_3O$
Mol. mass	:	299.75 g/mol
Routes	:	Oral, intramuscular

Chlordiazepoxide is a sedative/hypnotic drug and benzodiazepine which act on the brain and nerves (central nervous system) to produce a calming effect. It works by enhancing the effects of a certain natural chemical in the body (GABA). Main uses of Chlordiazepoxide includes Myasthenia gravis, Acute intoxication with alcohol, narcotics, or other psychoactive substances, Ataxia, Severe hypoventilation, Acute narrow-angle glaucoma, Severe liver deficiencies (hepatitis and liver cirrhosis decrease elimination by a factor of 2), Severe sleep apnea, Hypersensitivity or allergy to any drug in the benzodiazepine class.

Many people using this medication do not have serious side effects. Drowsiness, ataxia and confusion have been reported in some patients particularly the elderly and debilitated. Blood dyscrasias (including agranulocytosis), jaundice and hepatic dysfunction have occasionally been reported during therapy [7,8]. When Chlordiazepoxide treatment is protracted, periodic blood counts and liver function tests are advisable. But serious side effects occur are mental/mood changes, slurred speech, clumsiness, trouble walking, decreased/increased interest in sex, tremor, uncontrollable movements, facial or muscle twitching, trouble urinating, sleep disturbances. Drowsiness, dizziness, nausea, constipation, blurred vision, or headache may occur. Very few methods of determinations like HPLC [9,10,11], HPTLC [12] and Spectroscopy [13] and are so far reported**.** Most of them are methods of determination of the drug simultaneously with other CNS drugs like Amitryptyline,[14],Mebeverine Hydrochloride,Carvedilol and Hydrochlorothiazide [15].

MATERIALS AND METHODS

Materials

Working standard of Chlordiazepoxide was obtained from well reputed research laboratories. HPLC grade water, Methanol and OPA were purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system, PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system was used with Rheodyne manual sample injector (with switch 77251), Analytical column Chromosil C18 (250×4.6mm) and the electronic balance-DENVER (SI234). Manual Rheodyne injector with a 20 µL loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance.

Determination of wavelength of maximum absorbance

The standard solutions of Chlordiazepoxide were scanned in the range of 200 - 400 nm against mobile phase as a blank. Chlordiazepoxide showed maximum absorbance at 217nm and hence selected for determination of the drug.

Chromatographic equipment and conditions

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of CHLORDIAZEPOXIDE an isocratic PEAK HPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

The mobile phase consisted of Methanol:Acetonitrile:0.1%OPA 50:40:10v/v,(*p*H 4.7). Injections were carried out using a 20 µL loop at room temperature (20 + 2 °C) and the flow rate was 1 mL/min. Detection was performed at 217nm with 10 min runtime.

Standard and sample solutions

A 10 mg amount of Chlordiazepoxide reference substance was accurately weighed and dissolved in 10 mL mobile phase in a 10 mL volumetric flask to obtain 1000 ppm concentrated solution. Required concentrations were prepared by serial dilution of this solution.

A composite of 20 Chlordiazepoxide (Ebrium-10mg) tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Chlordiazepoxide was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 mL mobile phase was added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 140ppm.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation, the quality of the chromatograph was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤ 2.0 and theoretical plates >2500. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

Table.1 System suitability parameters of Chlordiazepoxide

API Concentration	140ppm
Mobile Phase	Methanol:Acetonitrile:0.1% OPA 50:40:10 (v/v)
Wavelength	217nm
Column	C_{18} Column
*p*H	4.7
Concentration	140ppm
Retention Time	3.58min
Run Time	10 min
Area	377118
Th. Plates	2844
Tailing Factor	1.44
Pump Pressure	11.7 MPa

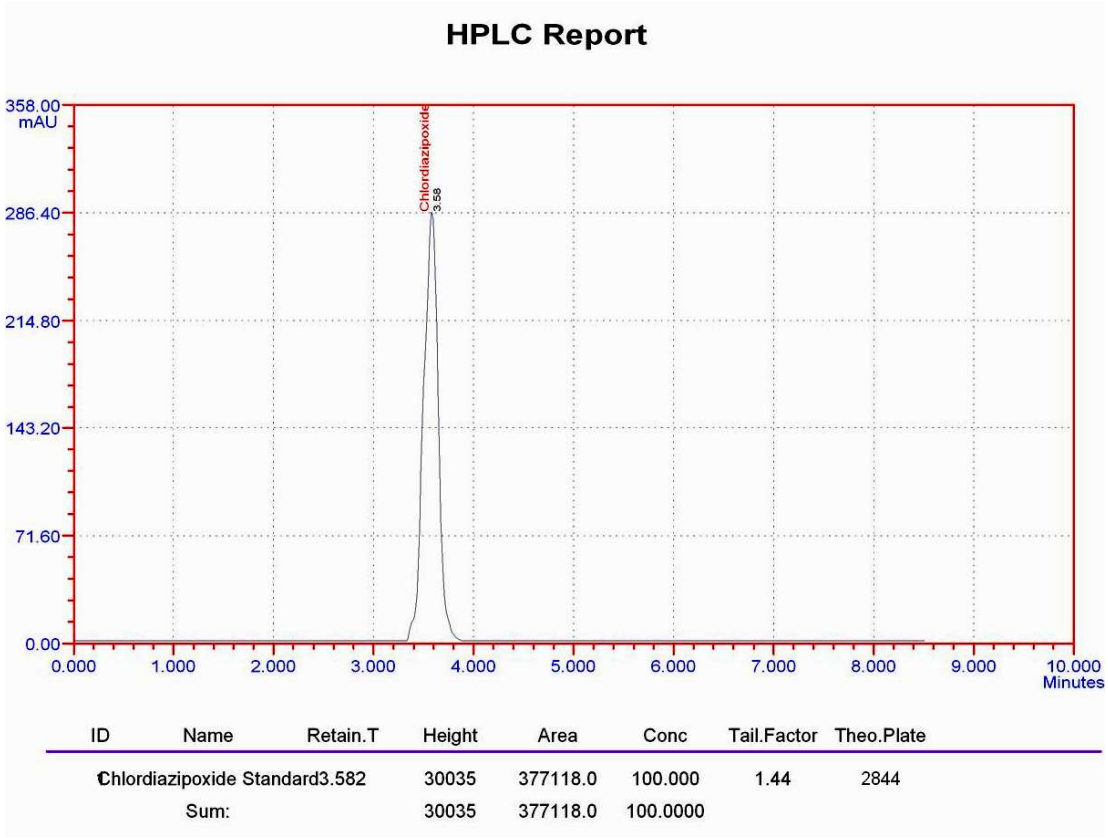

Figure 2: Standard chromatogram of Chlordiazepoxide

Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200ppm for Chlordiazepoxide. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was

y = 2444.05+ 2616x (r= 0.999). Linearity values are shown in Table 2.

Table.2: Linearity results of Chlordiazepoxide

S.No	Concentration (ppm)	Area
1	20	48336
2	40	102878
3	60	158868
4	80	206547
5	100	257365
6	120	292802
7	140	377118
8	160	413764
9	180	472454
10	200	520716
	Slope **Intercept** **CC**	2616.12 2444.05 0.99904

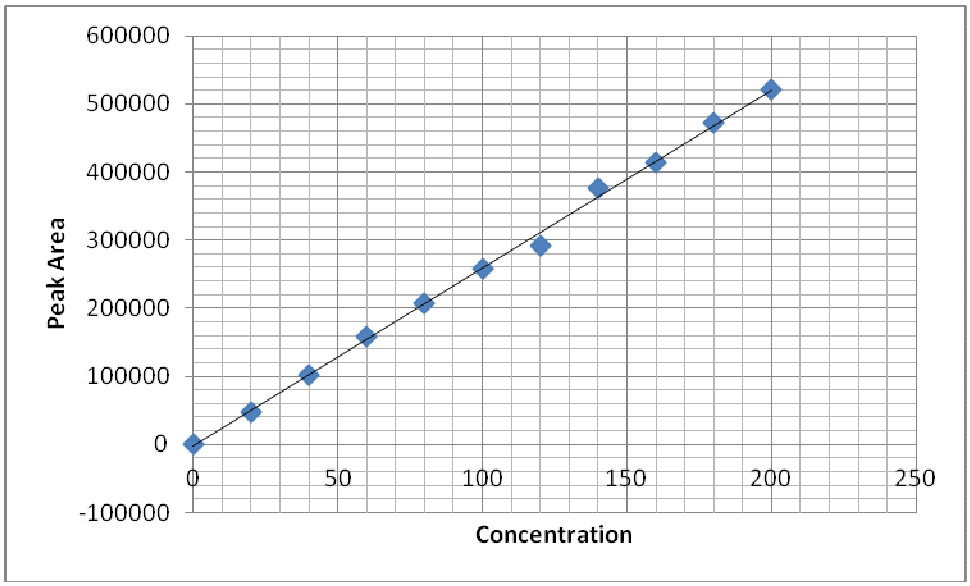

Figure 3: Calibration curve of Chlordiazepoxide

Precision

To study precision, six replicate standard solutions of Chlordiazepoxide (140ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3 and Table.4.

Table 3: Intraday Precision Results for Chlordiazepoxide

Sample (µg/ml)	Area
1	377118
2	372596
3	370177
4	372719
5	375463
6	374530
RSD	**0.65**

Table 4: Inter day Precision results of Chlordiazepoxide

Sample (µg/ml)	Area
1	381610
2	373849
3	385491
4	384265
5	385870
6	372908
RSD	**1.5**

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) ,the sample was dissolved by using Mobile phase and injected until peak was disappeared. After 2ppm dilution Peak was not clearly observed, based on which 2ppm is considered as Limit of Detection and Limit of Quantification is found to be 7 ppm.

Table.5: LOD and LOQ results of Chlordiazepoxide

Parameter	Measured Value
Limit of Quantification	7ppm
Limit of Detection	2ppm

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. The robustness study was performed by slight modification in flow rate of the mobile phase, composition of the mobile phase and wavelength of the detector. Chlordiazepoxide at standard concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above and the results were shown in table 6.

Table.6: Robustness results of Chlordiazepoxide

S.NO	Parameter	Change	Area	% of Change
1	Standard	……	377118	……
2	Mobile Phase	Methanol:ACN:0.1%OPA 55:35:10 45:45:10	 374994 379825	 0.56 0.71
3	*p*H	4.6 4.8	378909 377202	0.47 0.02
4	Wavelength	221nm 211nm	373488 373995	0.96 0.82

Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of a week. Ruggedness is expressed in terms of percentage relative standard deviation.

Sample	**CHLORDIAZEPOXIDE**
Concentration(in ppm)	140
Injection No.	**Area**
1	371314
2	373992
3	373109
4	379000
5	375016
6	379355
RSD	**0.86**

Table 8: Recovery results of Chlordiazepoxide

Level	Target Conc. (ppm)	Spiked conc. (ppm)	Final Conc. (ppm)	Conc. Obtained	% of Recovery
50%	40	20	60	58.9	98.2
	40	20	60	59.2	98.6
	40	20	60	59.1	98.6
100%	40	40	80	79.1	98.9
	40	40	80	79.7	99.6
	40	40	80	81.3	101.6
150%	40	60	100	99.2	99.2
	40	60	100	99.6	99.6
	40	60	100	98.2	98.2

Recovery

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e. 60ppm, 80ppm, 100ppm. The percent recovery was calculated and results are presented in Table 8. Satisfactory

recoveries ranging from 98.7 to 101.6 were obtained by the proposed method. This indicates that the proposed method was accurate. Results are given in table 8.

Formulation Analysis:
For assay 20 tablets of Chlordiazepoxide (Ebrium-10mg) were weighed and calculated the average weight. Accurately weighed and transferred the sample equivalent to 10mg of Chlordiazepoxide into a 10mL volumetric flask. Added diluent and sonicated to dissolve it completely and made the volume up to the mark with diluents, made homogeneous and filtered through 0.45µm filter. Further pippetted 1.4mL of the above stock solution into a 10mL volumetric flask and diluted up to mark with diluents and finally 140ppm was prepared and filtered through 0.45µm filter. An aliquot of this solution was injected into HPLC system. Peak area of Chlordiazepoxide was measured for the determination and the findings were presented in Table 9.

Table 9: Formulation analysis

Formulation	Brand name	Concentration	Amount found	%Assay
Chlordiazepoxide	Ebrium (10mg)	140ppm	138.75*	99.1

**mean of five determinations*

CONCLUSION

The proposed method for the assay of Chlordiazepoxide in capsules is very simple and rapid. It should be emphasized that it is isocratic and the mobile phase does not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. The method could effectively separate the drug from its products. Thus the method can be used for analysis of Chlordiazepoxide in formulations.

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