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Determination of Chlordiazepoxide in Pure Drug Samples, Pharmaceutical Dosage Forms and Environmental Wastewater Samples Using High Performance Liquid Chromatographic Method

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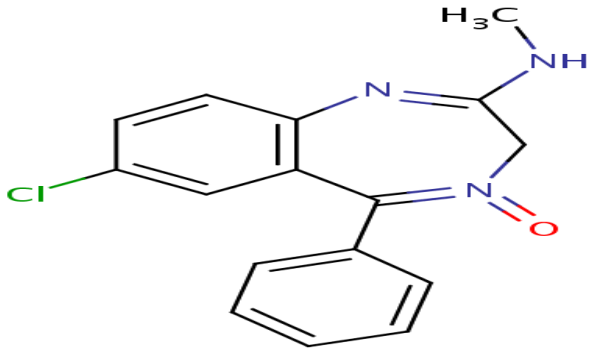
ABSTRACT

A simple, precise, rapid and accurate reverse phase high performance liquid chromatographic method (HPLC) has been developed for the determination of Chlordiazepoxide in pure drug samples, pharmaceutical dosage forms and environmental wastewater samples. Chromatography was carried out on supelcoC18 reversed-phase column(25cm x 4.6 mm. 5 µm).Using a mixture of methanol –acetonitrile- 0.05M KH_2PO_4 (40: 20:40 v/v/v) as a mobile phase. And pumped at a flow rate of 1.0 ml/min, the peaks were detected at 246 nm. Linearity was obtained in the concentration range of 0.01-0.20 mg/ml. Limit of detection (LOD) and limit of quantization (LOQ) were found 2µg/ml and 6µg/ml respectively. The method was statistically validated and RSD was found to be less than 1.8% indicating high degree of accuracy and precision of the proposed HPLC method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining Chlordiazepoxide in pure drug samples, pharmaceutical dosage forms, and environmental wastewater samples.

Key words: Chlordiazepoxide, HPLC, Wastewater

INTRODUCTION

Chlordiazepoxide, (Figure 1) is7-Chloro-*N*-methyl-5-phenyl-3*H*-1,4-benzodiazepin-2-amine 4-oxide.[1]



$C_{16}H_{14}ClN_3O$ 299.8

Figure 1: Chemical Structure of Chlordiazepoxide

It used as an anxiolytic, sedative-hypnotic, tranquilizer, and anti-depressant. It shares the actions of other benzodiazepines and is used for the management of anxiety disorders or for short-term relief of symptoms of anxiety and for the management of agitation associated with acute alcohol withdrawal [2-3].Several methods have been reported for the quantitative determination of Chlordiazepoxide in cludingtitrimetry [1],HPLC[4-8],gas chromatography(GC)[9], spectrophotometric[10-15], ion-selective electrodes [16]and voltammetry [17-18] .This paper reports a simple, sensitive and accurate new high performance liquid chromatographic(HPLC) method for determination of Chlordiazepoxide in pure form and pharmaceutical preparations.

MATERIALS AND METHODS

The chromatographic system consisted of an Shimadzu HPLC model LC-20AT with UV detector model SPD-20A and L_1(C18) Supelco column (25cm×4.6mm), 5 microns. HPLC conditions are given in Table1.

Table 1: HPLC conditions

Column	L1(C18)
Wavelength	246nm
Injection volume	20µl
Flow rate	1.0ml/min
Temperature	Ambient
Retention time	1.8
Mobile Phase	Methanol –acetonitrile- 0.05MKH_2PO_4 (40: 20 :40 v/v/v)

Reagents: All chemicals used were of analytical or pharmaceutical grade, high-purity water was used throughout and Chlordiazepoxide standard material was provided from AL-Hokamaa Company for pharmaceutical industries (HPI) Mosul-Iraq.

Chlordiazepoxide standard stock solution was prepared by dissolving accurately weighed quantity of 200mg of the drug in 100ml of 0.1 M hydrochloric acid(Final concentration, 2mg/ml). Working standard solutions in the range of (0.01-0.20 mg/ml) were prepared by dilution from this stock solution.

Recommended procedure

Chromatographic separation was achieved at ambient temperature on a reversed phase C18 column (25cm×4.6mm),5 microns. Using a mobile phase consisting of Methanol –acetonitrile- 0.05MKH_2PO_4 (40: 20:40 v/v/v) at flow rate 1.0ml/min, the detector wavelength was at 246nm.Calibration graph: Working standard solution equivalent to 0.01-0.2 mg/ml Chlordiazepoxide were prepared by appropriate dilution of standard solution with ethanol. 20µl aliquot of each solution was injected on to the column in a duplicate and the chromatograms were recorded. Calibration graph was constructed by plotting the mean peak area versus concentration of Chlordiazepoxide. The concentration of the unknown was read form the calibration graph or calculated from the regression equation derived from the concentration and peak area data.

Procedure for pharmaceutical preparations (tablets)

To minimize a possible variation in the composition of the tablet, the mixed content of 10 tablets, provided from AL-Hokamaa Company for pharmaceutical industries (HPI) Mosul-Iraq).). were weighed and grounded, then the powder equivalent to 10mg of Chlordiazepoxide in to 100ml volumetric flask, was added to about 50ml of 0.1 M hydrochloric acid and mixed well for 30 minute to dissolve, completed to the volume with 0.1 M hydrochloric acid , filtered and then determined the concentration of Chlordiazepoxide as described under recommended procedure.

Procedure for industrial wastewater samples

To demonstrate the practical applicability of the proposed method, real industrial wastewater samples from the state company for pharmaceutical industries (HPI) Mosul-Iraq were collected in polyethylene container cleaned with nitric acid, and filtered through filter paper No.41. Filtered samples were stored at 4 c^0 until analyzed which shows negative results, then the samples were spiked with the concentrations ranging from 20-180µg/ml of Chlordiazepoxide and determination of Chlordiazepoxide as described under recommended procedure. Calculate the percentage recovery using a calibration graph previously prepared.

RESULTS AND DISCUSSION

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and pharmaceutical products. The aim of this study was to develop a rapid HPLC method for the determination of Chlordiazepoxide in pure from, its pharmaceutical formulations and industrial waste water samples using the most commonly employed RPC18column with UV detection. The detection wavelength of 246 nm was chosen in order to achieve a good sensitivity for quantitative determination of Chlordiazepoxide in tablet dosage form. The mobile phase consisting of Methanol –acetonitrile- 0.05MKH_2PO_4 (40: 20 :40 v/v/v) offered a good separation at room temperature under these conditions using a flow rate of 1.0ml/min and retention time of 1.8 min as shown in the chromatogram, Figure2 . Under the described experimental conditions the analytic peak were well defined and free from tailing.

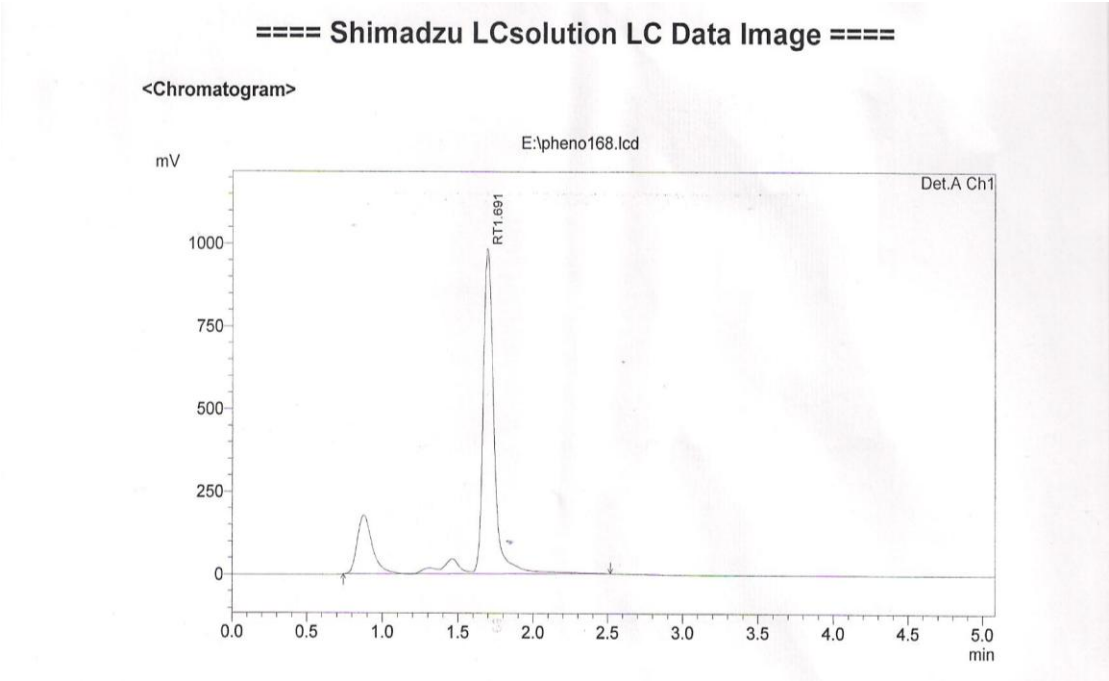


Figure 2: Typical chromatogram for Chlordiazepoxide 0.1mg/ml.

Chlordiazepoxide was determined by measuring the peak area. A plot of peak area against concentration gave a linear relationship (r=0.999) over the concentration range 0.01-0.2 mg/ml. Using regression analysis, the linear equation Y=44789x+0.8 was obtained where Y is the mean peak area, slope =44789and x is the concentration in mg/ml as shown in figure(3).Determination of limit of detection and limit of quantification(sensitivity).A series of dilute solutions were prepared in the range of 0.1%, 0.5% and 1% of the assay concentration (0.1mg/ml) using the standard solutions 20µl of each of the above solutionswereinjectedin6timesandthearea were calculated due to Chlordiazepoxide peak. The standard deviation of 6 injections for each concentration was calculated. The standard deviation at concentration 0 (blank) was calculated and this value was used for the calculation of the limit of detection and limit of quantification. The limits of detection (LOD) and quantification (LOQ) were calculated using the following equation: LOD= (3.3σ/s) and LOQ= (10 σ/s) where σ is the standard deviation of the response and s is the slope of the regression line [19]. Limit of detection (LOD) and limit of quantification (LOQ) were found 0.2 µg/ml and 0.6 µg/ml respectively. The results indicate that the method was sensitive enough to detect a concentration of 0.2µg/ml and able to quantify concentrations above 0.6µg/ml.

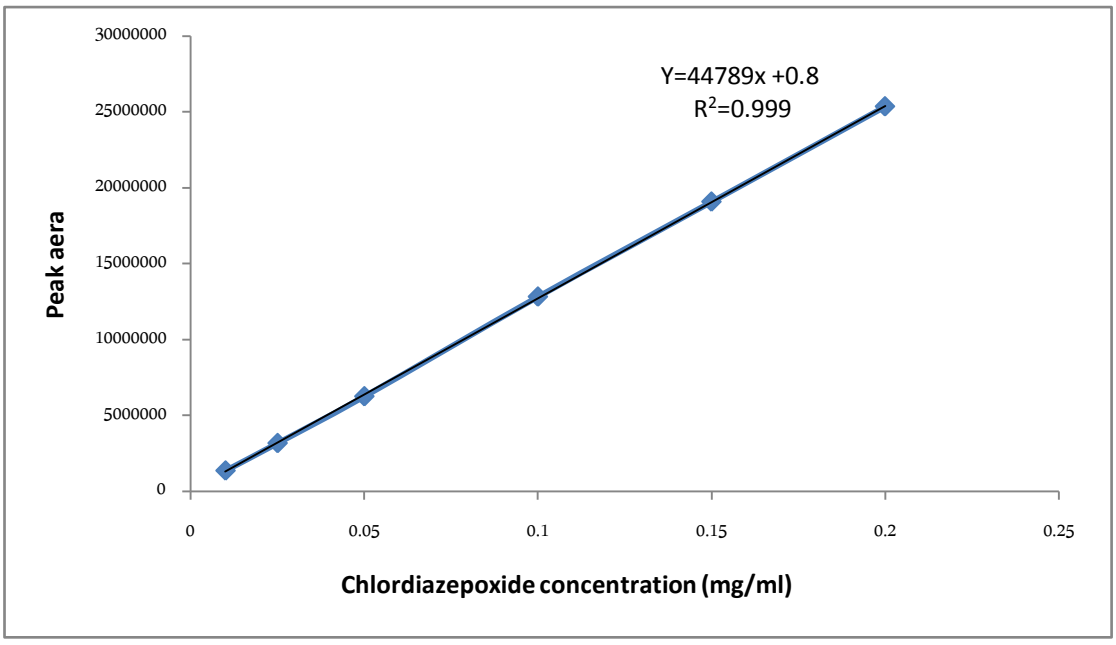


Figure 3; Calibration curve of Chlordiazepoxide.

Method precision

The precision of the method was established by carrying out the analysis of Chlordiazepoxide (n=10) using the proposed method .The low value of standard deviation showed that the method was precise. The result obtained were presented in Table2.

Table 2: Method precision.

Chlordiazepoxide concentration (mg/ml)	% Assay Mean(n=10)	%RSD of Assay (n=10)
0.025	0.0245	0.9
0.05	0.052	1.1
0.10	0.109	0.8
0.12	0.121	1.7

Method accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out at three different levels. The results of recovery studies were found to be satisfactorily high, mean recoveries being 100.3±1.0 (n=3) as shown in table3.

Table 3: Method accuracy.

Chlordiazepoxide Amount added (mg)	Amount found Mg)(	%Recovery n =3
0.025	0.0251	100.4
0.05	0.0498	99.6
0.1	0.101	101.0
Mean= 100.3 ±1.0		

Analytical application

The proposed method was successfully applied to the assay of Chlordiazepoxide in tablets and wastewater samples. No interfering peaks were found in the chromatogram, indicating that the excipients did not interfere with the estimation of the drug by the proposed HPLC method. The results obtained are presented in table 4, which reveals that there is close agreement between the results obtained by the proposed method and the label claim for the determination of Chlordiazepoxide in Pharmaceutical formulations and the results for wastewater samples, (Table 5) show that good agreement between results and known values indicated the successfully applicability of the proposed method for determination of Chlordiazepoxide in environmental wastewater samples.

Table 4: Determination of Chlordiazepoxide.

Pharmaceutical Formulations(HPI)	Label amount (mg)	Found* (mg)	% Recovery
Tablets(Librax-H)	5	5.025	100.5

* Mean value of ten determinations.

Table(5): Determination of Chlordiazepoxide in wastewater samples.

Wastewater samples	**Added(µg/ml)**	**Found*(µg/ml)**	**Recovery %(n=10)**
Industrial wastewater	20	.120	100.5
	60	59.6	99.33
	180	182.1	101.16

* mean value of ten determinations

CONCLUSION

In this study, a simple, rapid, accurate HPLC method was developed and validated for the determination of Chlordiazepoxide in pure drug samples, pharmaceutical dosage forms and environmental wastewater samples. The method was selective using L1 (C18) analytical column and applicable to pharmaceutical preparations. Thus the developed method is recommended for control throughout the entire manufacturing process of drugs as well as quality control of the finished product in view of its high recovery and precision.

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