AJPS, 2017, Vol. 17, No.2 Date of acceptance:20-9-2017

High Performance Liquid Chromatographic Method for the Determination of Chlordiazepoxide in Pharmaceutical Preparations Application to content uniformity testing

Nief Rahman. Ahmed

Department of Environmental Technology,College of Environmental University of Mosul,Mosul-Iraq

niefpharm@yahoo.com

Abstract:

A reverse phase high performance liquid chromatographic method (HPLC) has been developed for the determination of chlordiazepoxide in the pharmaceutical formulations . Separation was achieved using supelco C18 column(25cm x 4.6 mm. 5 µm). The mobile phase (ethanol), adjusted pH to 1.0 with 1N sulfuric acid and pumped at a flow rate of 1.0 ml/min . The peaks were detected at 310 nm. Linearity was obtained in the concentration range of 0.01- 0.20 mg/ml .The method was statistically validated and RSD was found to be less than 1.5 % 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, and pharmaceutical dosage forms.

Keyword: HPLC, Chlordiazepoxide, Pharmaceutical formulations.

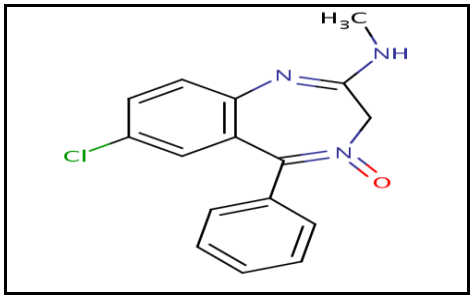
تقدير كلورديازيبوكسايد باستخدام كروماتوعرافيا السائل ذات الأداء العالي في المستحضرات الصيدلانية وتطبيق الطريقة لاختبار الاتساق

الخلاصة:

تم اختبار طريقة كروماتوغرافيا السائل ذات الأداء العالي لتقدير عقار كلورديازيبكسايد في حالته النقية وفي بعض مستحضراته الصيدلانية (الحبوب) حيث تم استخدام عمود supelco C18 (25cm x 4.6 mm. 5 µm) و تم استخدام الطور الناقل (الايثانول) وتم تثبيت درجته الحامضية على 1 باستخدام 1عياري حامض الكبريتيك وبسرعة جريان 1مل\دقيقة وتم القياس عند الطول الموجي 310 نانومتر وأمكن قياس الكميات التي تتراوح بين 0.20 -0.01 ملغم\مل وتم تطبيق المعادلات الإحصائية لمعرفة صلاحية الطريقة ووجد أن معامل الانحراف القياسي للطريقة النسبي اقل من1. 5 % مما يدل على ضبط ودقة الطريقة نظرا لتكرارية وبساطة الطريقة ودقتها العالية فقد تم استخدامها لتقدير كلورديازيبكسايد بشكله النقي وفي مستحضر الحبوب.

Introduction :

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



C16H14ClN3O 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-termrelief 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 including titrimetry[1]. HPLC [4-8] , gaschromatography (GC) [9] , spectrophotometry [10-15] , ion-selective electrodes [16] and voltametry [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 L1(C18)Supelco column (25cm×4.6mm), 5 microns. HPLC conditions are given in Table-1.

Column	Table-1 : HPLC conditionsL1(C18)
Wavelength	310nm
Injection volume	20µl
Flow rate	1.0ml/min
Temperature	Ambient
Retention time	1.69
Mobile Phase	Ethanol(acidify to pH1by 1N H_2SO_4

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 ethanol[BDH] (Final concentration , 2 mg/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 ethanol(adjusted pH to 1.0 with 1N sulfuric acid); at flow rate 1.0ml/min, The detector wavelength was at 310nm.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 ethanol and mixed well for 30 minute to dissolve, completed to the volume with ethanol, filtered and then determined the concentration of chlordiazepoxide as described under recommended procedure.

Results and discussion

The development of HPLC methods for the determination of drugs has received conceder-able 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 and its pharmaceutical formulations the most commonly employed RP C18 column with UV detection. The detection wavelength of 310 nm was chosen in order to achieve a good sensitivity for quantitative determination of chlordiazepoxide in tablet dosage form. The mobile phase consisting of ethanol and the pH adjusted to 1.0 with dilute sulfuric acid offered a good separation at room temperature under these conditions using a flow rate of 1.0ml/min and retention time of 1.69 min as shown in the chromatogram, Figure[2] . Under the described experimental conditions the analyte peak were well defined and free from tailing .

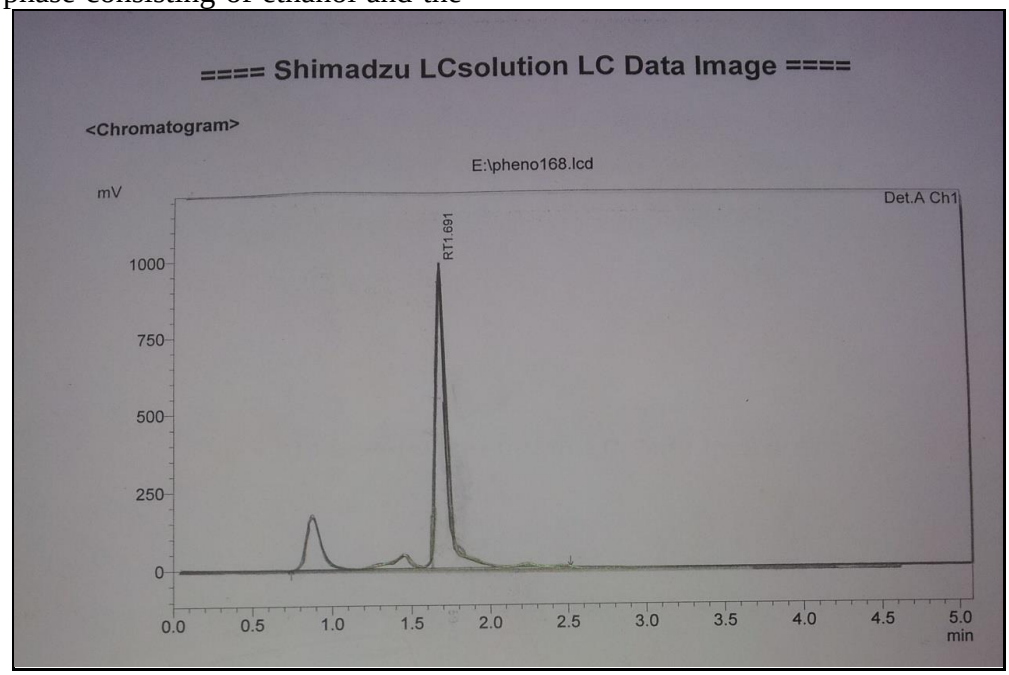


Figure-2: Typical chromatogram for chlordiazepoxide 0.1mg/ml

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 =44789 and x is the concentration in mg/ml as shown in figure-3.

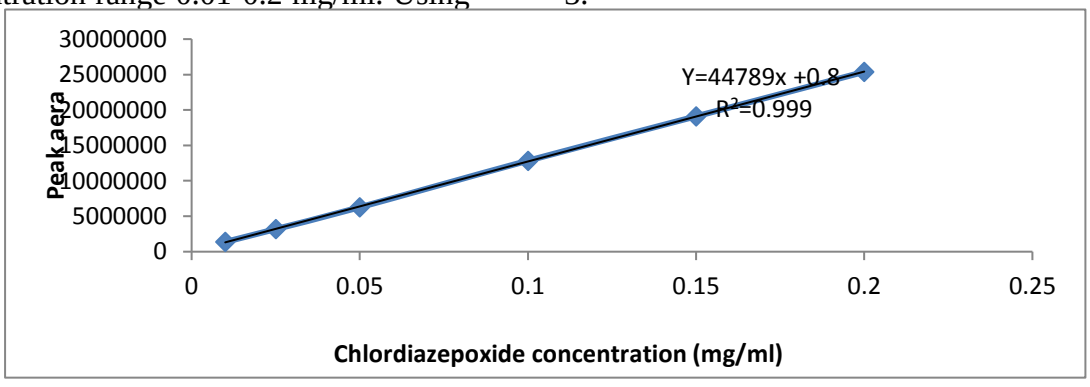


Figure-3; Calibration curve of chlordiazepoxide.

The chromatogram of pharmaceutical preparation (tablets) 0.1 mg / ml chlordiazepoxide was shown in Figure [4]. 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 solutions were injected in 6 times and the area 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

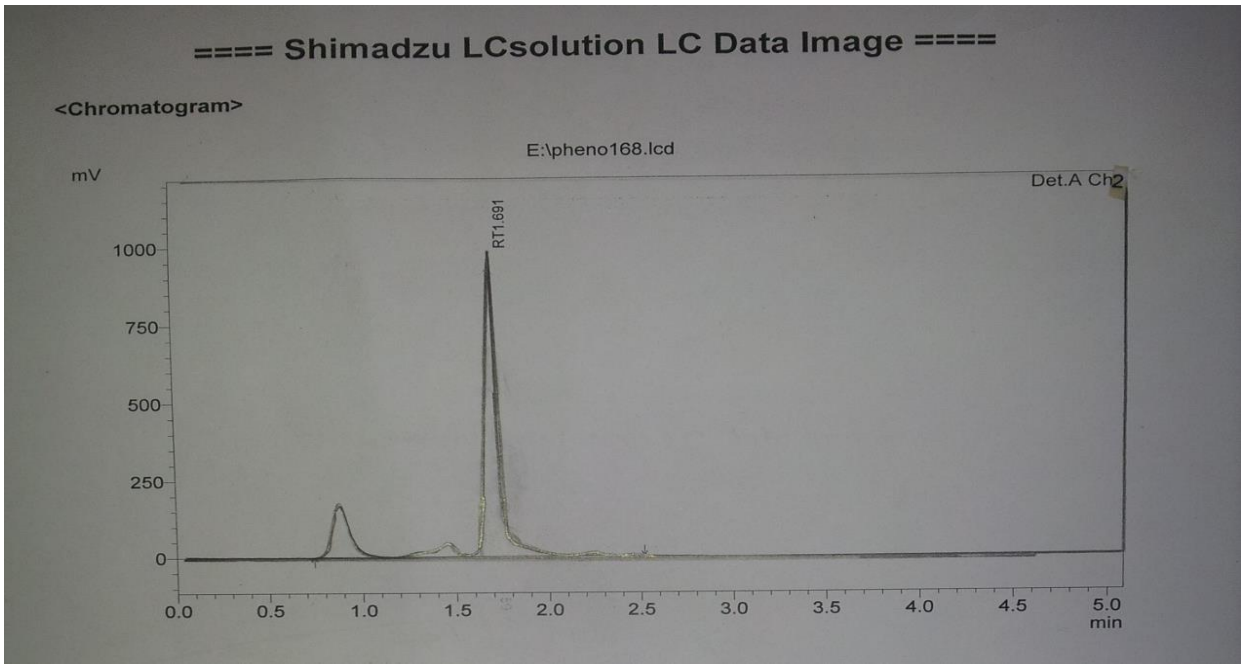


Figure-4 : Typical chromatogram for chlordiazepoxide 0.1mg/ml in tablets

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 0.2 µg/ml and able to quantify concentrations above 0.6 µg/ml.

Method precision and accuracy

The precision of the method was established by carrying out the analysis of chlordiazepoxide (n=6) using the proposed method .The low value of standard deviation showed that the method was precise. The result obtained were presented in Table-2

Table -2 :Method precision

Chlordiazepoxide concentration (mg/ml)	Assay (mg/ml) Mean(n=6)	%RSD of Assay (n=6)
0.025	0.0249	0.9
0.05	0.0505	1.1
0.10	0.109	0.8
0.12	0.121	1.4

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 table[3] Table{2 and 3}indicates a satisfactory precision and

accuracy could be obtained with the proposed method.

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. The result of analysis for pharmaceutical formulations Table-4 which reveals that there is close agreement between the results obtained by the proposed method and label claim.

Table-4 : Determination of chlordiazepoxide.

Pharmaceutical Formulations(HPI)	Label amount (mg)	Found* (mg)	% Recovery
Tablets(Librax-H)	5	5.01	100.2

* Mean value of ten determinations

Application of the proposed method to content uniformity

Content uniformity or the uniformity of dosage unit was defined as the degree of uniformity in the amount of active substance among dosage units. The risk assessment strategy underlying content uniformity testing is the assumption that some pre-specified limits exist where safety and efficacy outcomes may change if content uniformity fails. The proposed method proved to be suitable for the content uniformity test, where a great number of assays on individual tablets are required. Data presented in table-5 indicate that the proposed method can accurately and precisely quantitate chlordiazepoxide in its commercially available tablets. The mean percentage with (RSD) of the labeled claim found in ten tablets was (0.321%) which falls within the content uniformity limits specified by the USP 30 [20].

Table-5 : Content uniformity testing of chlordiazepoxide tablets

Parameter	% label claim
Tablet NO.1	100.2
Tablet NO.2	99.9
Tablet NO.3	100.2
Tablet NO.4	100.2
Tablet NO.5	99.8
Tablet NO.6	100.5
Tablet NO.7	99.8
Tablet e NO.8	100.4
Tablet NO.9	100.5
Tablet NO.10	99.5
Mean(X)	100.1
%RSD	0.321
Max. allowed unit value[20]	±15%

Conclusion:

In this study, a simple, rapid, accurate HPLC method was developed and validated for the determination of chlordiazepoxide in pure form and tablets. 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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