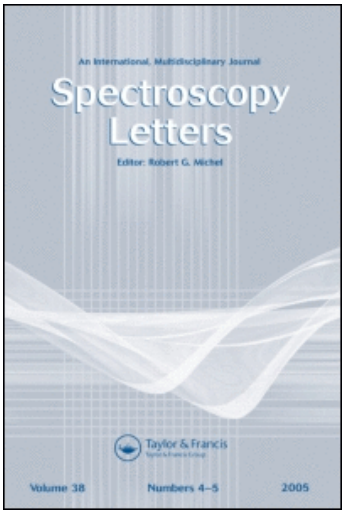

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ANALYSIS OF CHLORDESMETHYLDIAZEPAM BY THREE DIFFERENT TECHNIQUES

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ANALYSIS OF CHLORDESMETHYLDIAZEPAM BY THREE DIFFERENT TECHNIQUES

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ABSTRACT

Three simple, rapid, and accurate techniques have been developed for the determination of chlordesmethyldiazepam. The first one depends on the spectrophotometric determination of the orange azodye resulting from the coupling of thymol with the acid induced hydrolysis product of chlordesmethyldiazepam after conversion to the corresponding diazonium salt. The second is the application of quantitative densitometry for the simultaneous determination of chlordesmethyldiazepam in the presence of its degradation product. While the third technique implies HPLC resolution efficiency in the determination of chlordesmethyldiazepam in the presence of its degradation product.

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The validity of the proposed procedures were proved using the standard addition technique and laboratory prepared mixtures of the drug and its degradation product(s). The proposed procedures were successfully applied for the drug analysis in Tablet form.

Key Words: Benzodiazepines; Chlordesmethyldiazepam; Benzophenone; Diazometric determination; Stability; Densitometry; HPLC

INTRODUCTION

Chlordesmethyldiazepam (2-chloro-(2′-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepine-2-one) is one of the benzodiazepine compounds (Fig. 1). It is of clinical interest as an anti-anxiety agent. It is formulated in Tablets known as E.N for the control of anxiety[1].

It has been determined by high performance liquid chromatography. Acetonitrile/methanol/water mixture was used as the eluent system and the column used was Merck 10 micron Lichrosolv RP18, 15 cm length, dimensions $150 \times 4.6\,mm^2$.

EXPERIMENTAL

Apparatus

– Shimadzu, UV-1601 PC, UV-Visible Spectrophotometer.
– Shimadzu-Dual wavelength flying spot CS-9000 densitometer.
– Precoated TLC plates, silica gel 60 F_{254} 20×20 cm, 0.25 mm thickness (Alugram).
– Hamilton® syringe 25 µl.

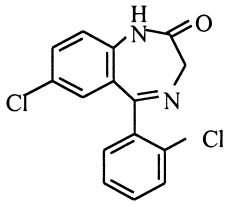

Figure 1. Chlordesmethyldiazepam (2-chloro-(2′-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepine-2-one).

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– Perkin Elmer Liquid chromatograph with Perkin Elmer series 410 LC pump and Perkin Elmer LC-235 Diode Array Detector. The stationary phase was Phenomenex®. Prodigy 5 µ ODS, 100 A., dimension: 150 × 4.6 mm internal diameter, 5 µm particle size.

Pure Samples

Chlordesmethyldiazepam (RAVIZZA, ITALY) was kindly supplied by AL-KAHIRA Pharm & Chem. Ind. Co., Cairo, Egypt. Its purity found to be (99.99 ± 0.49) according to the manufacturer method[2].

Market Samples

E.N Tablets batch number 810834. Each Tablet claimed to contain 0.5 mg of chlordesmethyldiazepam manufactured by Al. KAHIRA Pharm & Chem. Ind. Co., Cairo, Egypt., under license of RAVIZZA-MILANO-ITALY.

Reagents

All reagents and solvents used were of analytical and spectroscopic grades: methanol, thymol, chloroform, methanol HPLC grade, hydrochloric acid, potassium hydroxide and sodium nitrite.

Diazometric Method

Drug Standard Solution for Linearity

Chlordesmethyldiazepam stock solution (200 µg ml^{-1}) in methyl alcohol. It is prepared by refluxing a quantity of 10 mg of chlordesmethyldiazepam in 10 ml of 10% (v/v) hydrochloric acid solution for 20 minutes, the mixture is cooled and transferred quantitatively into a 50 ml volumetric flask, completed to volume with methyl alcohol.

Identification of the Wavelength of Maximum Absorbance

Transfer separately aliquot portions containing 1000 µg of chlordesmethyldiazepam from its stock solution (200 µg ml^{-1}) into a couple of 25 ml volumetric flasks. Treat one of the two flasks with 4 ml of 0.2% sodium

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nitrite freshly prepared solution and stand for about 5 minutes then add 0.8 ml of 0.2% thymol in 10% potassium hydroxide solution. Complete the volume with distilled water. The other flask is to be used as a blank. Record the absorbance of both solutions after 20 minutes against reagent blank solution (Fig. 2).

Construction of Calibration Curve

Experiment

Transfer accurately aliquoted portions equivalent to (200–1000 μg) of chlordesmethyldiazepam from its stock solution (200 μg ml^{-1}) into a series of 25 ml volumetric flasks, add to each 4 ml of 0.2% sodium nitrite freshly prepared solution and stand for about 5 minutes then add 0.8 ml of 0.2% thymol in 10% potassium hydroxide solution. Complete the volume with distilled water.

Blank

Reflux 10 ml of 10% (v/v) hydrochloric acid solution for 20 minutes, cool and transfer quantitatively into a 50 ml volumetric flask. Complete to the volume by methyl alcohol.

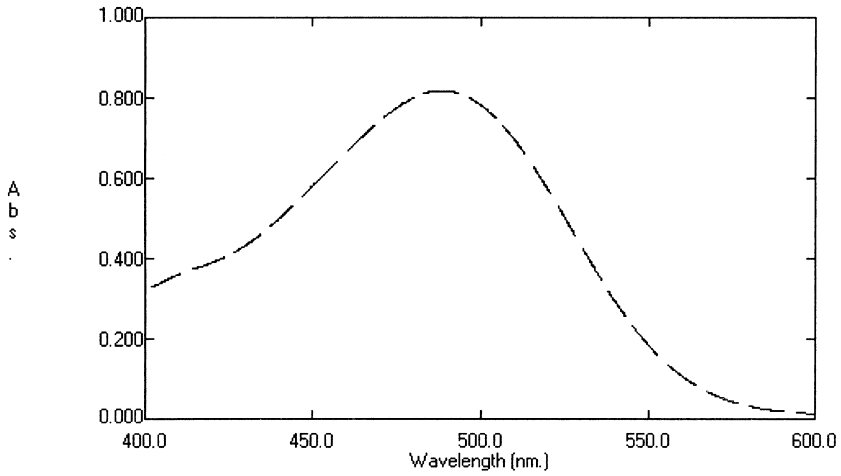

Figure 2. Absorbtion spectrum of the azodye product (40 μg ml^{-1}) (———) showing a maximum absorbance at 488 nm.

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Record the absorbance of the orange azodye produced after 20 minutes at its maximum wavelength 488 nm against a similarly treated blank of reagent.

Construct the calibration curve by plotting the concentration versus the absorbance and compute the regression equation (1).

Assay of E.N Tablets

Weigh accurately and powder 30 Tablets. Weigh an amount of powder equivalent to 10 mg chlordesmethyldiazepam in a conical flask and extract with 30 ml methyl alcohol. Filter to another flask and re-extract the residue twice with 20 ml of methyl alcohol and refilter. Evaporate the alcoholic extract to dryness and add 10 ml of 10% (v/v) hydrochloric acid. Reflux the mixture for 20 minutes, cool and transfer quantitatively into 50 ml volumetric flask completing the volume with methyl alcohol. Transfer 2 ml of this solution to a 25 ml volumetric flask, add 4 ml of 0.2% sodium nitrite solution and then proceed as under construction of calibration curve starting from "stand for...". Results obtained are shown in Table 3.

Stability Indicating Densitometric Method

Drug Standard Solution for Linearity

– Chlordesmethyldiazepam stock solution ($200\,\mu g\,ml^{-1}$) in methyl alcohol.

Prepared Mixtures

– Chlordesmethyldiazepam stock solution ($3\,mg\,ml^{-1}$) in methyl alcohol.
– Degradation product stock solution ($3\,mg\,ml^{-1}$) in methyl alcohol prepared by acid hydrolysis of chlordesmethyldiazepam with 0.1 N hydrochloric acid. The degraded product was extracted with chloroform after neutralization and the solvent was evaporated under vacuum. It yields yellow crystals of 2-amino-5,2′-dichlorobenzophenone having a melting point of 200°C[1].

From both stock solutions, aliquot portions are accurately transferred into a series of 10 ml volumetric flasks to prepare different mixtures

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containing (20–80%) of the degradation product completing the volume with methyl alcohol.

Construction of Calibration Curve

From stock solution of chlordesmethyldiazepam (200 μg ml^{-1}), apply 2.5, 5, 7.5, 10, 12.5, 15 μl equivalent to (0.5–3 μg) to a thin layer chromatographic plate using a 25 μl syringe. Spots are spaced 2 cm apart from each other and from the bottom edge of the plate. Develop the plates in a chromatographic tank previously saturated with the developing mobile phase, chloroform/methyl alcohol 42:3 (v/v) by ascending chromatography through a distance of 10 cm at room temperature. Dry the plate then detect the spots under the UV lamp and scan under the following conditions:

Photo mode: Reflection
Scan mode: Zigzag
Result output: chromatogram and area under the peak.
Swing width: 16 mm
Wavelength: 228

Record the area under the peak, construct the calibration curve by plotting the concentration against the peak area and compute the corresponding regression equation (2).

Assay of Prepared Mixtures

Apply 10 μl of the prepared mixtures to a silica gel plate and proceed as under construction of calibratuon curve starting from "spots are spaced...". Record the area under the peak. Calculate the concentration of the drug from the regression equation (2). Results obtained are shown in Table 1.

Assay of E.N Tablets

Weigh accurately then powder 30 Tablets, weigh an amount of powder equivalent to 10 mg in a conical flask then add 30 ml of methyl alcohol and stir for 20 minutes. Filter into a 50 ml volumetric flask and re-extract the residue with another 10 ml of methyl alcohol then refilter. Complete to volume with methyl alcohol.

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Table 1. Determination of Chlordesmethyldiazepam in Presence of Its Degradation Product in Laboratory Prepared Mixtures by the Densitometric Technique

	Chlordesmethyldiazepam		
Mixture No.	Taken (μg/spot)	Found (μg/spot)	Recovery %
1	3.000	2.997	99.916
2	2.400	2.395	99.797
3	1.800	1.812	100.688
4	1.200	1.224	102.025
5	0.600	0.595	99.203
Mean ± S.D	—	—	100.326 ± 1.087

Apply 5 μl from the prepared solution to a silica gel plate and proceed as under construction of calibration curve staring from "spots are spaced...". Results obtained are shown in Table 3.

Stability Indicating HPLC Method

Drug Standard Solution for Linearity

– Chlordesmethyldiazepam stock solution (100 μg ml^{-1}) in methanol.

Prepared Mixtures

– Chlordesmethyldiazepam stock solution (100 μg ml^{-1}) in methyl alcohol.
– Degradation product stock solution (100 μg ml^{-1}) in methyl alcohol prepared as under densitometric method.

From both stock solutions, aliquot portions are accurately transferred into a series of 10 ml volumetric flasks to prepare different mixtures containing (20–80%) of the degradation product completing the volume with methanol.

Construction of Calibration Curve

Transfer accurately aliquot portions equivalent to (5–25 μg) of chlordesmethyldiazepam from its stock solution (100 μg ml^{-1}) into a series

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of 10 ml volumetric flasks. Complete to volume with methanol. The samples are chromatographed under specified chromatographic conditions. The detector is set at 240 nm wavelength and 25 nm bandwidth. The mobile phase is prepared by mixing methanol and water in a ratio 65:35 v/v respectively. The solvent is filtered using 0.45 μm membrane filters and degassed by ultrasonic vibrations. The flow rate is set at 1.5 ml/min. All determinations are performed at ambient temperature. The samples are also filtered using 0.45 μm membrane filters and injected (20 μl) with a 25 μl Hamilton® analytical syringe. Report the peak area for each sample, construct the calibration curve by plotting the concentration versus the peak area and compute the corresponding regression equation (3).

Assay of Prepared Mixtures

Five laboratory prepared mixtures are chromatographed under specified chromatographic procedures. Record the area under the peaks and calculate the concentration of chlordesmethyldiazepam using the regression equation (3). Results obtained are represented in Table 2.

Assay of E.N Tablets

Weigh accurately and powder 30 Tablets. Weigh an amount of powder equivalent to 10 mg of chlordesmethyldiazepam into a conical flask. Add 50 ml of methanol and shake for 20 minutes then filter the solution into a 50 ml volumetric flask washing the residue twice with methanol. Complete to volume with methanol.

Table 2. Determination of Chlordesmethyldiazepam in Presence of Its Degradation Product in Laboratory Prepared Mixtures by HPLC Method

	Chlordesmethyldiazepam		
Mixture No.	Taken (μg)	Found (μg)	Recovery %
1	25	24.911	99.643
2	20	20.156	100.780
3	15	15.068	100.453
4	10	9.825	98.255
5	5	4.992	99.835
Mean ± S.D	–	–	99.793 ± 0.975

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Table 3. Statistical Analysis of the Results Obtained by the Proposed Methods and the Manufacturer Method for the Analysis of E.N Tablets 0.5 mg-Batch No.:810834

	Recovery % of Chlordesmethyldiazepam			
Preparation	Diazometric Method	Densitometric Method	HPLC Method	Manufacturer Method
1	101.330	99.440	98.736	99.612
2	101.330	98.193	99.079	100.024
3	101.064	100.331	98.461	100.376
Mean ± S.D	101.241 ± 0.154	99.322 ± 1.074	98.759 ± 0.310	100.004 ± 0.383

From this stock solution, transfer aliquot portions equivalent to 100 µg into a 10 ml volumetric flask. Complete the volume with methanol and carry out the previously mentioned chromatographic conditions. Record the peak area for each sample. Calculate the concentration of each from the regression equation. Results obtained are shown Table 3.

RESULTS AND DISCUSSION

Diazometric Method

Diazotization is a very well known reaction for compounds containing a primary amino group and has been abundantly used for the spectrophotometric determination of such compounds through coupling of their diazonium salts with suitable coupling agents such as thymol (substituted phenol)[3,4].

The proposed diazometric method for the determination of chlordesmethyldiazepam depends on its ability to form a diazonium salt through its acid hydrolysis product 2-amin-5,2′-dichlorobenzophenone produced by action of 0.1 N hydrochloric acid[1], which possess a typical primary amino group. This diazonium salt is readily liable to coupling with the aromatic system of thymol.

The present study describes the use of this coupling reaction of 2-amino-5, 2′-dichlorobenzophenone as diazonium salt with thymol for the determination of chlordesmethyldiazepam in bulk powder and pharmaceutical preparations. Diazocoupling reaction takes place at room temperature. The orange coloured azodye produced exhibited a maximum absorbance at 488 nm.

Table 4. Application of the Standard Addition Technique to the Analysis of E.N Tablets by the Proposed Methods

	Diazometric Method		Densitometric Method		HPLC Method	
Preparation	Added (μg/ml)	Recovery %	Added (μg/spot)	Recovery %	Added (μg/ml)	Recovery %
E.N Tablets	8	102.287	0.5	99.322	5	101.851
0.5 mg	12	101.170	1	102.411	10	101.873
Batch	16	98.750	1.5	101.302	15	100.370
No.: 810834	20	98.787	2	100.035		
	24	99.344				
Mean ± S.D	–	100.068 ± 1.584	–	100.767 ± 1.368	–	101.365 ± 1.368

The study of the effect of different reagents volumes revealed that the optimum absorbance and linearity between the absorbance of the formed azodye and drug concentration were obtained when 4 ml of 0.2% sodium nitrite solution and 0.8 ml of 0.2% thymol in 10% potassium hydroxide solution were used.

The maximum colour intensity was attained after 20 and it was stable for about one hour.

Linear relationship was obtained between the absorbances of the reaction product and the concentrations of the parent drug in the range of (8–40 $\mu g\,ml^{-1}$).

The regression equation calculated was found to be:

$$Y = 0.0235X - 0.106 \quad \text{with a correlation coefficient } 0.9999 \qquad (1)$$

where Y is the absorbance at 488 nm and X is the concentration of the drug in μg/ml.

By applying the regression equation (1), it was possible to determine chlordesmethyldiazepam in pure form with mean recoveries of (99.915 ± 1.013) at 488 nm as shown in Table 5.

The proposed method was successfully applied for the analysis of chlordesmethyldiazepam in its dosage form and results obtained are shown in Table 3.

The validity of the procedures was further assessed by applying the standard addition technique. Results obtained are shown in Table 4.

Based on the forgoing results, the following reaction pathway could be proposed:

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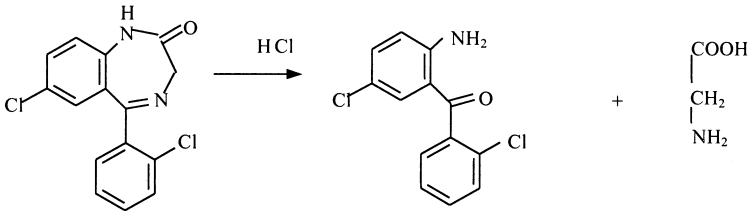
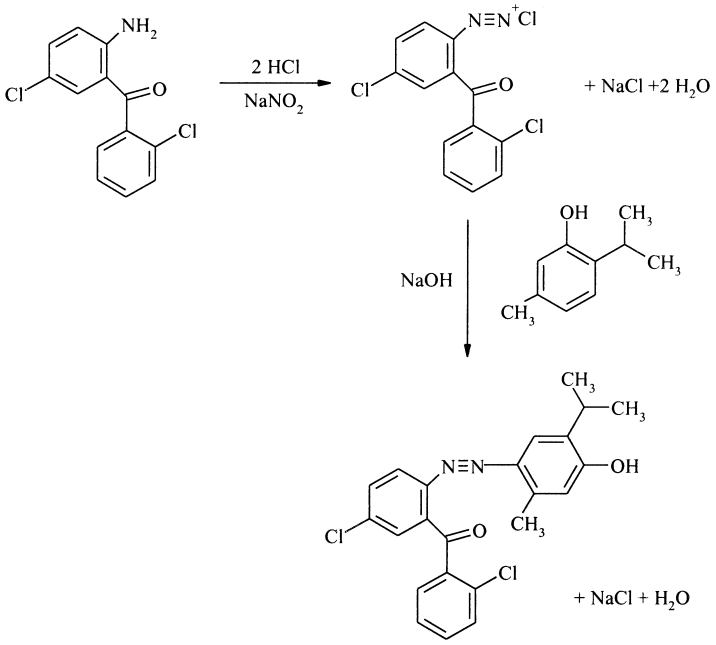


Chlordesmethyldiazepam 2-amino-5,2'-dichlorobenzophenone Glycine



Densitometric Method

Since the degraded product has no anxiolytic activity. This work is dealing with the application of a stability indicating densitomtric technique for the simultaneous determination of chlordesmethyldiazepam in the presence of its degradation product. The method is based on the

difference in the R_f values of the two compounds since R_f is 0.74 and 0.90 for both chlordesmethyldiazepam and its degradation product, respectively. Chloroform/methyl alcohol 42:3 (v/v) was used as a developing system. Complete separation of the two compounds indicates that each compound can be scanned separately on the same plate at the corresponding wavelength without any interference from the other compound.

By applying this technique, a linear correlation was obtained between the area under the peak and the corresponding concentrations of chlordesmethyldiazepam in the range of (0.5–3 μg/spot).

The regression equation calculated was found to be:

$$Y = 5.656\,X + 1.0387 \quad \text{with a correlation coefficient } 0.9999 \qquad (2)$$

where Y is the integrated peak area and X is the concentration of the drug in μg/spot.

By applying the regression equation (2), it was possible to determine chlordesmethyldiazepam in pure form with mean accuracies of (99.872 ± 1.093) as shown in Table 5.

The proposed densitometric technique was applied in the determination of chlordesmethyldiazepam in laboratory prepared mixtures containing up to 80% of the degradation product with mean accuracies of (100.326 ± 1.087) as shown in Table 1.

It was possible by applying the proposed method to determine chlordesmethyldiazepam in Tablet form and satisfactory results were obtained as shown in Table 3.

Table 5. Statistical Analysis of the Results Obtained by the Proposed Methods and the Manufacturer Method for Chlordesmethyldiazepam in Pure Powdered Form

	Chlordesmethyldiazepam			
Item	Diazometric Method	Densitometric Method	HPLC Method	Manufacturer Method
Mean ± S.D	99.915 ± 1.013	99.872 ± 1.093	100.020 ± 1.018	99.998 ± 0.491
n	5	5	5	6
Variance	1.026	1.195	1.036	0.241
Student's t	0.16 (1.83)	0.24 (1.83)	0.04 (1.83)	–
F	4.26 (5.19)	4.96 (5.19)	4.30 (5.19)	–

The Figures in parenthesis are the corresponding theoretical values (at P = 0.05).

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The validity of the suggested densitometric method was further assessed by applying the standard addition technique (Table 4).

HPLC Method

This study aims to develop a simple stability indicating HPLC assay for the analysis of chlordesmethyldiazepam in bulk material and in pharmaceutical preparations.

Preliminary studies were carried out to investigate the optimum percentage of methanol and water. Best peak shape was obtained with methanol/water 65:35 (v/v) with a retention time of 4.52 ± 0.02 minutes for chlordesmethyldiazepam and of 9.58 ± 0.02 minutes for its degradation product.

Linear relationship was obtained between the peak area and the concentrations of the drug in the range of ($5–25\,\mu g\,ml^{-1}$).

The regression equation calculated was found to be:

$$Y = 2.9108 \times 10^6 \times X + 0.82 \times 10^6 \quad \text{with a correlation coefficient } 0.9999 \tag{3}$$

where Y is the peak area and X is the concentration of the drug in $\mu g/ml^{-1}$.

By applying the regression equation (3), it was possible to determine chlordesmethyldiazepam in pure form with mean recoveries of (100.020 ± 1.018) as shown in Table 5.

The selectivity of the proposed method was checked by the analysis of different samples of intact chlordesmethyldiazepam by the proposed HPLC method in the presence of varying amounts of its degradation product. The mean percentage found was 99.793 ± 0.975 indicating that the method is not affected by the presence of any percentage of degradation product as shown in Table 2.

The proposed method was found applicable for the determination of chlordesmethyldiazepam in its dosage form and results obtained are shown in Table 3.

The validity of the proposed method was checked by applying the standard addition technique. Results obtained are shown in Table 4.

Table 5 shows statistical comparison of the results obtained by the proposed methods and the manufacturer method[2] for the determination of chlordesmethyldiazepam in pure powdered form. It is evident that the calculated t and F values are less than the theoretical ones indicating that there is no significant difference between the suggested procedures and the manufacturer one with respect to accuracy and precision.

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