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SPECTROPHOTOMETRIC DETERMINATION OF CERTAIN BENZODIAZEPINE DRUGS

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ABSTRACT

A rapid adequate spectrophotometric method was developed for the quantitative determination of diazepam, nordiazepam, oxazepam and chlordiazepoxide in pure form and in pharmaceutical preparations. The method is based on the hydrolysis of the above mentioned drugs in acid medium for 20 minutes in a boiling water bath. The formed amines were condensed with p-dimethylaminocinnamaldehyde in acidic methanol to form pink coloured product with absorption maxima at 500 and 530 nm for diazepam and the other drugs respectively. Beer's law was obeyed over the range of 0.5-10 ug ml^{-1} for all studied drugs. The produced colour is stable for 2 hours for diazepam and at least for 24 hours for the other studied drugs. The method is simple, sensitive and particularly suited for routine analysis of these drugs.

INTRODUCTION

A variety of techniques have been used for the determination of benzodiazepines; such as non aqueous titration[1], ultraviolet spectrophotometry[2-4] and colorimetry[5-8].

Spectrophotometric determination of certain benzodiazepine drugs

Recently published gas liquid chromatography and high pressure liquid chromatographic methods for benzodiazepines are concerned with their analysis in biological media[9-11].

New procedures were reported for the determination of these drugs in pharmaceutical dosage forms include HPLC[12-14] fluorimetry[15,16] and polarography[17-19]. However, most of these methods are not suitable for moderate to large scale drug quality programs.

The use of p-dimethylaminocinnamaldehyde (PDAC) as a colorimetric agnet for primary aromatic amines in acidic solution was reported previously[20]. This reagent is advantageously employed in place of the usual aldehydes such as p-dimethylaminobenzaldehyde and vanillin, since the resulting Schiff bases are red. This report presents a simple, rapid and sensitive method for the determination of four benzodiazepine drugs; namely; diazepam nordiazepam, oxazepam and chlordiazepoxide in pure form and in pharmaceutical preparations using PDAC.

EXPERIMENTAL

Apparatus

Ultraviolet-visible spectrophotometer, PM2 DL (Zeiss, West Germany) was used.

Materials

Pharmaceutical grade diazepam, nordiazepam(N-demethyldiazepam), oxazepam, chlordiazepoxide and chlordia-

zepoxide HCl were obtained as gifts from various manufacturers and were utilized as working standards. PDAC (Sigma, USA) was used as supplied. All other chemicals and solvents were of analytical grade. Various pharmaceutical preparations were purchased from the local market including tablets, ampoules and drops.

Reagents:

1- P-Dimethylaminocinnamaldehyde, 0.1% w/v in methanol
2- Hydrochloric acid, 2 and 4 N

Preparation of Standard Solutions

Diazepam, nordiazepam and oxazepam solutions, 1 mg ml^{-1} of each drug in 4 N HCl.

Chlordiazepoxide and chlordiazepoxide HCl solution, 1 mg ml^{-1} of each drug in 2 N HCl

Preparation of Sample Solutions

Tablets

Weigh and powder 20 tablets. Transfer an accurately weighed quantity of the powder equivalent to 25 mg of the corresponding drug to 25 ml volumetric flask. Dilute to about 20 ml with 2 N HCl for chlordiazepoxide and 4 N HCl for diazepam, and shake for 15 minutes. Complete to volume with the same acid and shake well.

Filter , discard the first portion of the filtrate. The clear solution obtained is the assay solution.

Injections and Drops

Transfer to a 10 ml volumetric flask an accurately measured volume of injections or drops equivalent to about 10 mg of diazepam or nordiazepam. Complete to volume with 4 N HCl and mix well. This is the assay solution.

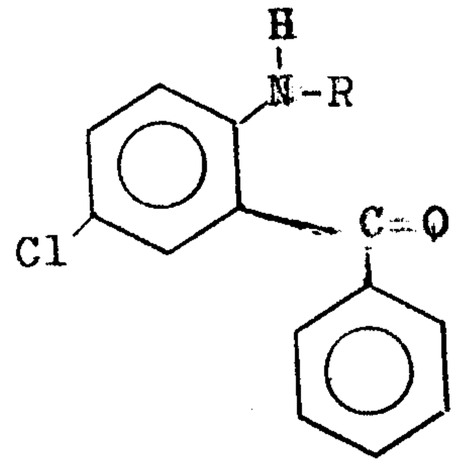
Procedure

Into 10 ml volumetric flask, transfer 10 ml of the standard or the sample solution and heat in a boiling water bath for 20 minutes. Cool to room temperature under running water and complete to volume if it is required with 2 N HCl for chlordiazepoxide and 4 N HCl for diazepam, nordiazepam and oxazepam. Dilute 1 ml of each hydrolysed solution with methanol to 10 ml. To 1 ml of this solution, add 3 ml of 0.1% PDAC solution and complete to volume with methanol in 10-ml volumetric flask. Measure the absorbance of the resulting solution at 500 nm after 20 minutes for diazepam and at 530 nm immediately for the other drugs against a blank similarly treated using 1.0 ml of acidified methanol (1 ml 2 N or 4 N HCl completed to 10 ml with methanol) instead of standard or sample solution.

Calculate the concentration of benzodiazepine drug by reference to a calibration graph obtained by assaying suitable standards by the method described.

RESULTS AND DISCUCCION

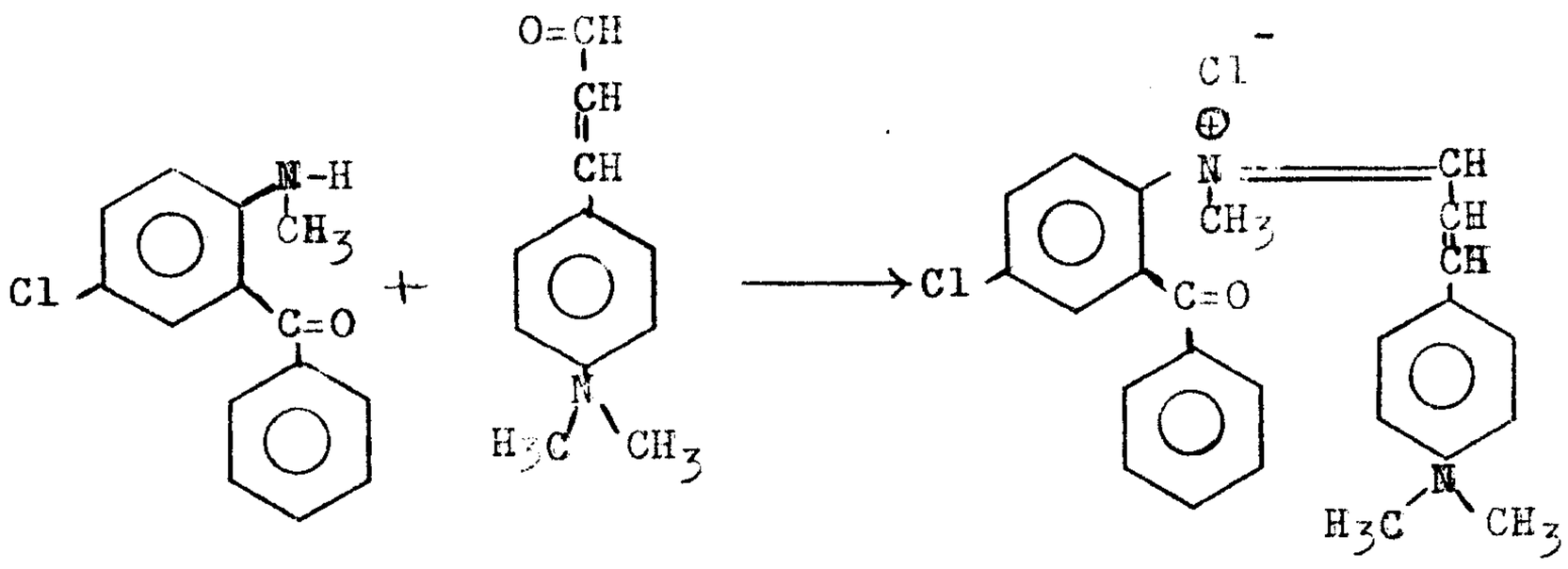
Diazepam, nordiazepam, oxazepam and chlordiazepoxide on acid hydrolysis produced a substituted benzophenone[21].



Where R= CH_3 for diazepam
H for nordiazepam, oxazepam and chlordiazepoxide.

In this investigation, the substituted benzophenone formed was condensed with PDAC to form a higly

coloured chromogen. The reaction of the hydrolyzed product of nordiazepam, oxazepam, and chlordiazepoxide with PDAC is simply a Schiff base formation. On the other hand the reaction between the hydrolyzed product of diazepam and PDAC is supposed to take place through the condensation of the protonated secondary amino group of the hydrolyzed product with the aldehyde group of the reagent to produce an iminium salt[22] (Scheme 1).



PDAC Iminium salt

(Scheme 1)

The interaction of the hydrolyzed product of all studied drugs with PDAC in acidified methanol produced a red colour showing an absorption peak at 500 nm for diazepam and 530 nm for other drugs (Fig. 1).

Optimum normality of HCl used in the hydrolytic step was selected on the basis of ensuring solubility of benzodiazepines as well as their hydrolytic products and at the same time giving maximum colour intensity on interaction with PDAC. It is obvious from Table 1, that optimum normality of HCl is 2 for chlordiazepoxide and 4 for the other investigated drugs. Twenty minutes heating in a boiling water bath for all studied drugs was found to be quite sufficent to ensure complete hydrolysis and hence maximum colour intensity (Table 2).

The absorbance of the resulting solutions was found to increase with increasing PDAC concentration up to 0.4 mg ml^{-1} in the final solution (Table 3). However, blank solutions containing reagent concentration higher than 0.3 mg ml^{-1} are dark in colour. The use of 3.0 ml volume of 0.1% PDAC solution was considered satisfactory for the drug concentration levels proposed in this assay.

The colour reached maximum intensity after 20 minutes and remained stable for 2 hours for diazepam. For other drugs the colour developed immediately and was stable for at least 24 hours.

The dilution of the interaction coloured products of all studied drugs by different solvents, namely ,

methanol, ethanol, isopropanol, dioxane, and 1,2-dichloroethane show no significant effect on the position of λ_{max}, while the intensity of absorption is affected. Methanol and 1,2-dichloroethane were found to be the best solvents as they gave the highest absorption. Methanol was used as diluent in this work for availability.

Under the proposed experimental conditions, Beer's law was obeyed for all drugs and a typical linear regression correlation was obtained (Table 4). The reproducibility of the procedure was determined by running replicate samples, each containing 5 ug ml^{-1} of chlordiazepoxide in the final test solution. At this concentration level, the relative standard deviation for 10 determinations was 0.44%.

The proposed method can be used as stability indicating assay for all the studied drugs by analyzing each drug before and after hydrolysis. the difference gives the amount of intact drug.

P-Dimethylaminocinnamaldehyde gives colour reaction with primary amines. However; in local market, each benzodiazepine drug is commercially available only in single drug component. So the problem of interference did not arise.

The developed method was successfully applied to

the quantitative determination of the investigated benzodiazepines in bulk materials and commercially available preparations. Furthermore; the proposed method was compared with the Bp 1980 method for the analysis of chlordiazepoxide tablets. Table 5 illustrates the effectiveness of the developed method and the good agreement between the results of both procedures.

Table 1: Effect of normality of HCl used in the hydrolysis of certain benzodiazepines on the colour intensity

HCl (N)	Absorbance+ of Diazepam at λ_{max} 500 nm	Nordiazepam	Oxazepam at λ_{max} 530 nm	Chlordiazepoxide HCl
0.5	a	a	b	0.167
1.0	a	a	b	0.408
2.0	a	a	b	0.556
3.0	0.422	0.615	0.538	0.453
4.0	0.505	0.780	0.608	0.439
5.0	0.410	0.460	0.348	0.374

* By heating in a boiling water bath for 20 minutes. Final concentration for all drugs, 5.0 ug ml^{-1}

a The hydrolyzed products remain undissolved.
b Oxazepam remains undissolved.
\+ Average of 3 experiments.

Table 2: Effect of reaction time in hydrolytic step of certain benzodiazepines on the intensity of colour formed with PDAC

Time (min)	Absorbance+ of Diazepam at λ_{max} 500nm	Nordiazepam	Oxazepam at λ_{max} 530nm	Chordiazepoxide HCl
10	0.266	0.473	0.432	0.250
10	0.414	0.494	0.450	0.413
20	0.505	0.760	0.606	0.556
30	0.506	0.750	0.607	0.555
40	0.507	0.760	0.603	0.556

\+ Average of 3 experiments.
Final concentration for all drugs 5.0 ug ml^{-1}

Table 3: Effect of p-dimethylaminocinnamaldehyde (PDAC) concentration on colour intensity

Volume of 0.1% PDAC solution(ml)	Absorbance+ of Diazepam at λ max 500 nm	Chlordiazepoxide HCl at λ max 530 nm
1.0	0.325	0.223
1.5	0.400	0.312
2.0	0.445	0.403
2.5	0.486	0.500
3.0	0.508	0.556
3.5	0.558	0.596
4.0	0.600	0.630

\+ Average of 3 experiments
Final concentration, 5.0 ug ml^{-1}

Table 4: Spectral characteristics of hydrolyzed benzodiazepines PDAC chromogens

Drug	λ_{max}	ξx10	Linear calibration range ug ml^{-1}	Slope	Intercept	Correlation coefficient
Diazepam	500	2.97	0.5-8.0	0.077	0.012	0.999
Nordiazepam	530	4.30	0.5-6.0	0.117	-0.001	0.999
Oxazepam	530	3.49	0.5-8.0	0.113	0.003	0.999
Chlordiazepoxide HCl	530	3.73	0.5-10	0.100	0.006	0.999

Table 5: Determination of some benzodiazepines in bulk materials and in pharmaceutical preparations

Preparation and manufacturer	*Proposed method*		
	Found[a] % ±SD	Drug added mg	Recovery[a] % ±SD
Diazepam			
Powder (Roche)	100.1±0.42		
Calium Tablets (Arab Co.) 2 mg/tab.	100.5±0.55	5/tab.	99.1±0.63
Valium 10 ampoule(Roche) 10 mg/2ml	99.6±0.37	10/tab.	101.1±0.25
Nordiazepam			
Powder (Ravizza)	99.9±0.44		
Madar drops (Arab Co.) 0.5%	101.1±0.22	5/ml	100.5±0.51
Chlordiazepoxide			
Powder (Roche)	99.9±0.35		
Libran-5 tablets[x](Memphis) 5 mg/tab.	99.8±0.41	5/tab.	100.2±0.39
Libertan-10 tablets[*](Memphis) 10 mg/tab.	100.1±0.60	10/tab.	100.5±0.53

x BP 1980 method 100.2%

* BP 1980 method 99.9%

a Average of 3 determinations.

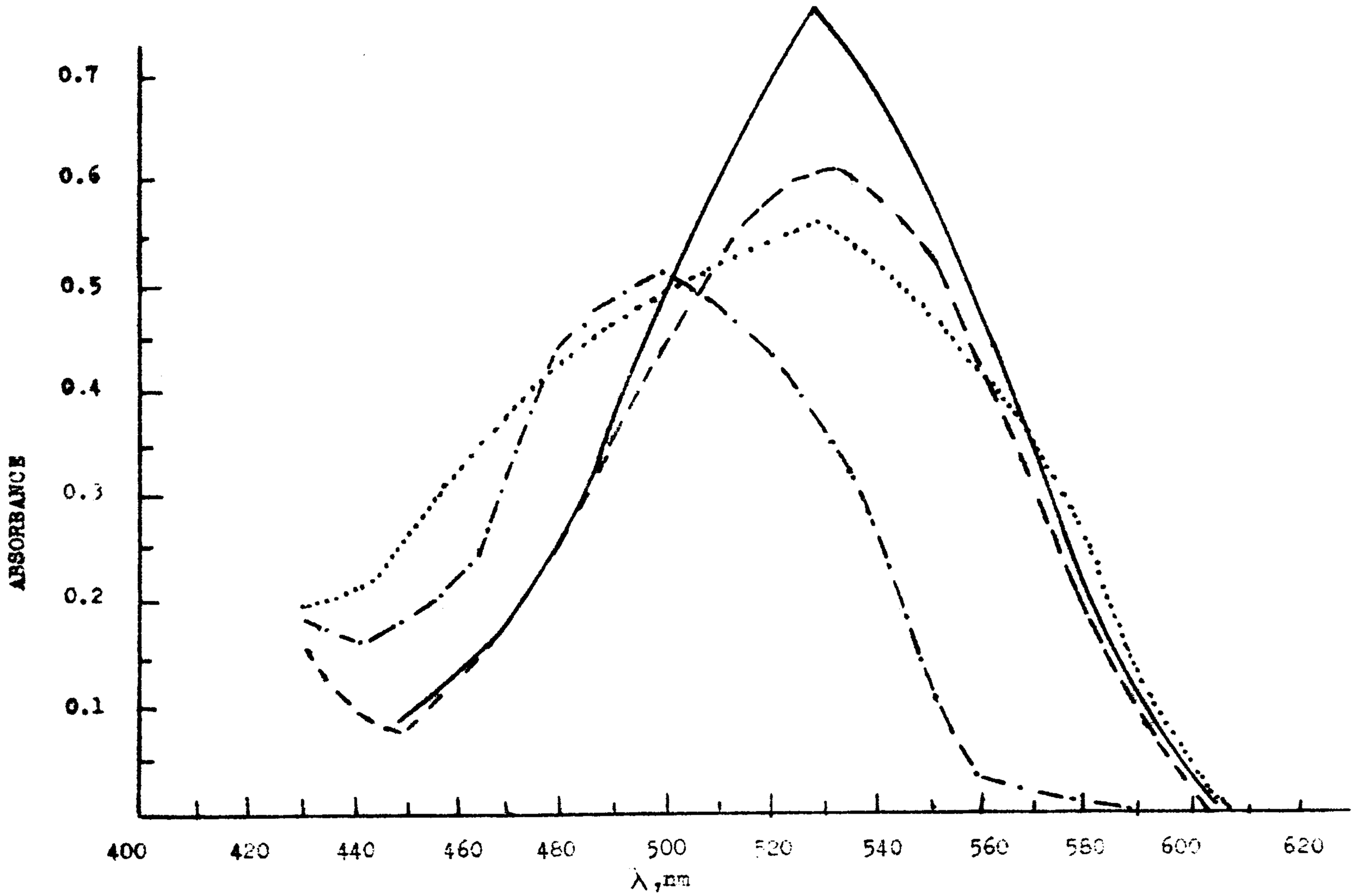


Fig. 1: Absorption spectra of hydrolyzed benzodiazepine-PDAC Chromogens: (—·—·—)diazepam,(————)nordiazepam,(-----)oxazepame and(·····)chlordiazepoxide, concentration for all drugs 5 µg ml^{-1}

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طريقـــــة طيفيـــة لتعيين بعض البنزوديازبينـــــات

سلوى رزق الشابورى ـ مديحة بخيت سيدهم

كليـــــة الصيـــــدلة ـ كليـــة الصيـــدلة ـ جـــامعـــة اسيـــــــوط

هـــذا البحـــث يحـــتوى عـــلى طريقـــة سهـلة لتعيين الـديازيبـــــــام النورديـــازيبـــام ، الاكسازيبـــام وكلورديازيبوا كسيد سـواء فى حالتهم النقية أو المستحضـرات الطبيـــة .

وهذه الطريقة تعتمـد على حلمأة هذه الادويـة الى أمينـات فى حمـام مـــائى عنـــد درجـــة الغليـــان لمـــدة ٢٠ دقيقـــة . وبتكثيـف الامين النـاتج مـــن حلمأة هـــذه العقـــاقير مع مقـــابل ثنائى ميثيـــل أمينوسينـاميك الدهيدفـــــى وسـط ميـــثانولى محمض يتكون لـون أحمـر لـه درجـة أمتصاص قصوى عنـد موجة طولـهـا ٥٠٠ نم للـديازيبـــام ، ٥٣٠ نم لبقيـــة العقـاقيـــــر .

وهـــذا اللـــون النـــاتج يتبـع قـــانون بيير من ٥ر ـ ١٠مكجم لكل عقار .

وقـد وجـد أن اللون النـاتج ثابت لمـدة ساعتين فى حالـة الديـازيبـام ولمـــدة ٢٤ ســـاعة لبقيـــة العقـــاقيـــر .

وقـــد وجـــد أن هـــذه الطـــريقة سهـلة وحساسة وصـــالحة للتحليـل الـــروتينى لهـــذه العقـاقيـــــير . سـواء فى المادة الخام او فى بعض المستحضرات الصيدلية .

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