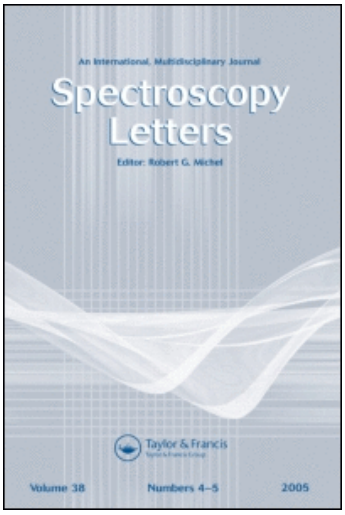

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Stability - Indicating Method For The Determination of Bromazepam And Delorazepam Via Proton Magnetic Resonance Spectroscopy

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Abstract

A new, simple proton magnetic resonance method (^{1}HNMR) for the rapid and direct quantitation of intact bromazepam and delorazepm in presence of their acid - induced degradation products is presented. DMSO - d_6 is used as a solvent and maleic acid as internal reference standard. The resonance peak of the two hydrogens of the methylene group of each drug near 4.2 ppm is integrated as a measure for the drug in comparison to the sharp singlet of maleic acid at about 6.25 ppm .

The proposed procedure gives accurate and reproducibe results when applied for the anlaysis of both drugs either per se, in mixtures with their degradation products or in their tablets.

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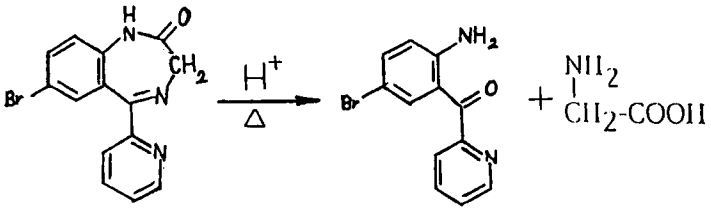
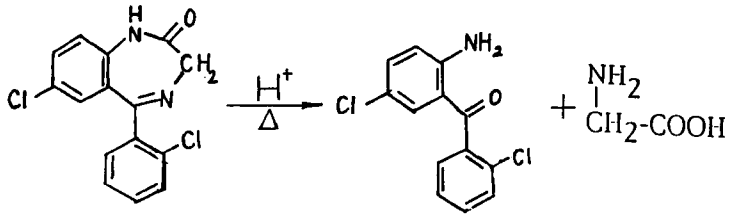
Introduction

The psychotropic benzodiazepines - which were originally considered to have less dependence potential - have got into criticism due to their increased abuse. Among them are bromazepam (I_a) and delorazepam (II_a) , which produce - through acid hydrolysis - the corresponding benzophenone derivatives (I_b and II_b) , together with glycine.[1-4]



Ia Ib glycine



IIa IIb

Several analytical techniques have been described for the determination of bromazepam, including chromatography[5,6] electrochemistry[7,8] and spectrophotometry [9,11] in addition to the recent nonaqueous titration adopted by B.P. (1993) [12] .

Few methods were reported for the assay of delorazepam mainly by chromatography [13,14] and polarography. [15]

The main object of the present work is to quantitate bromazepam and delorazepam in presence of their degradation products, using ^{1}HNMR.

Experimental

Apparatrus

Jeol - Ex 270 MHz and Varian Chemini 200 MHz, NMR spectrometers were used throughout this work.

Materials and reagents

All solvents and reagents used were of A.R. grade. Maleic acid (British Drug house), deuterated dimethyl sulfoxide (DMSO - d_6 , 99.7% isotopic purity, SIC Stohler istop chemicals) and trifluoroacetic acid (Winlab, UK, 99%) were used.

Bromazepam reference standard ; batch no. 9400004-329, Kindly supplied by Amoun Pharmaceutical Industries Co . (APIC) Cairo, Egypt. Its purity was checked by TLC[3], and was found to be 99.98 ± 0.46 % according to the B.P. (1993) method[12] . Standard delorazepam; Kindly supplied by kahira pharm. and chem. Ind. Co. Cairo, Egypt. Its purity was checked by TLC [4] and was found to be 100.05 ±0.97 % according to the manufacturer's method [16] .

Calmepam tablets, 1.5 mg and 3 mg, and E.N. tablets, 0.5 mg, were purchased from the local market.

Procedure

1. Authentic drugs' powders

Accurately weigh 5 -25 mg of I_a or II_a into small glass - stoppered test tube, add 1ml of DMSO - d_6 containing 8 mg. ml^{-1} maleic acid and stopper the tube . Mix well, then transfer 0.5 ml of the clear solution into an NMR tube. Run the ^{1}HNMR spectrum taking care to adjust spin rate to eleminate side bands especially in the range of interest. Reference all peak field position to DMSO - d_6 at 2.5 ppm, measure the integrals of the sharp singlet at about 4.2

ppm and 6.25 ppm for each drug and maleic acid , respectively. three times and get the average of each.

Calculate the weight of each drug using the following equation : (17)

$$Wd = \frac{Hs}{Hd} \cdot \frac{Md}{Ms} \cdot \frac{Id}{Is} \cdot Ws$$

Where : W = weight (mg. ml^{-1})

H = number of protons within signal

M = molecular weight

I = integral of signal (mm)

The subscripts "s" and "d" stand for the internal standard and the drug, respectively .

The equation could be written as :

$$W\ I_a = \frac{2}{2} \cdot \frac{316.2}{116.04} \cdot \frac{I_d}{I_s} \cdot 8$$

$$W\ II_a = \frac{2}{2} \cdot \frac{305.2}{116.04} \cdot \frac{I_d}{I_s} \cdot 8$$

2. Mixtures of intact and degraded drugs

(A) Preparation of laboratory degraded drugs[1,5] - Pipette 2-8 ml of the drugs' solutions (2.5 mg. ml^{-1}) in 6 N H_2SO_4 , or 6N HCL for bromazepam or delorazepam , respectivey, into a small flask and complete to 10 ml with the corresponding acid. Reflux for 2.5 hrs. in boiling water bath for I_a, or reflux for 1.25 hrs. on a hot plate (~ 150 ^{o}C) for II_a . Adjust the degradate solution of each

drug to pH 8-9 with 6 N NaoH (using universal indicator test paper) and transfer quantitatively into a separating funnel. Extract three times, by shaking each time for 10 min. with 15 ml diethyl ether. Collect the etherial extracts and evaporate to dryness at about 40 °C in the same small beaker. Dry the residue in a vacuum desiccator over sulfuric acid for 30 min .

N.B. The degradate of each drug was tested both by TLC using silica gel 60 F_{254} plates and a mobile phase of chorofrom - acetone (80:20)[3,4], and by the proposed ^{1}HNMR method . The results obtained by both techniques revealed the absence of any undegraded drugs.

(B) Validity testing of the procedure for the analysis of mixtures of intact and degraded drugs - Into a series of beakers containing 5- 20 mg of each laboratory degraded drug, accurately weigh 20-5 mg. of the pure intact drug. Add to each beaker 1ml of DMOS - d_6 containing 8 mg. ml^{-1} malcic acid. Proceed as under "1-Authentic drugs' powders" starting from " Mix well, then transfer 0.5 ml of the clear solution ....." .

3- Application to dosage forms; calmepam and E .N. tablets

Weigh, finely powder and mix thoroughly not less than 100 tablets. Accurately weigh a protion of the powder equivalent to 5-25 mg of I_a and 7.5 - 25 mg of II_a into a 50 ml volumetric flask. Extract three times, each with 20 ml $CHCl_3$, by shaking thoroughly for 15 min. , filter into small beaker through filter paper wetted with $CHCL_3$, wash the residue twice, each with 5 ml $CHCl_3$ and evaporate the combined extracts and washings almost to dryness in a 25 ml beaker on a hot plate at about 50 - 60 °C. Dry the residue in vacuum desiccator over sulfuric acid for 60 min. , then add 1ml DMSO - d_6 containing 8 mg. ml^{-1} maleic acid, and proceed as under "1- Authentic drugs' powders" starting from "Mix well then transfer 0.5 ml of the clear solution....."

Results and discussion

The choice of a solvent which would easily and quantitatively dissolves a large quantity of the two drugs without any interference in the integration region of interest (between 4-5 ppm) was not easy. Difficulties were especially encountered with bromazepam (I_a) which is poorly soluble in most organic solvents except methanol in which it is moderately soluble. Although delorazepam is freely soluble in ethanol, methanol, acetone , DMSO and chloroform, yet, it was difficult to choose a suitable deuterated available solvent that dissolves both the drug and the internal standard without any interference.

The ^{1}HNMR spectra of I_a and II_a in DMSO - d_6 using maleic acid as internal standard are shown in figs. 1 and 3. All signals, measured in delta - scale are referenced to DMSO - d_6 whose singlet is positioned at 2.5 ppm . These spectra are characterized by a sharp, well defined singlet near 4.2 ppm corresponding to the two methylene protons of each drug . This singlet has been choosen for the quantitative determination of each of the two drugs, by comparing its integral value with that of a known weight of maleic acid, whose down field resonance peak appeared at 6.25 ppm corresponding to its two equivelant vinylic protons. This allowed a more reliable and uncomplicated analysis. Neverless, the other resonance peaks appearing down field between 6.8-9 ppm, due to the aromatic protons, together with the singlet at10.7 - 10.8 ppm, due to the amide proton, are useful in the identification and checking the purity of both drugs.

The acid hydrolysis of I_a and II_a results in the formation of the benzophenone derivatives; 2 - amino 5- bromobenzoylpyridine (I_b) and 2-amino - 5,2 - dichlorobenzophenone (II_b), respectively, together with glycine [1-4].

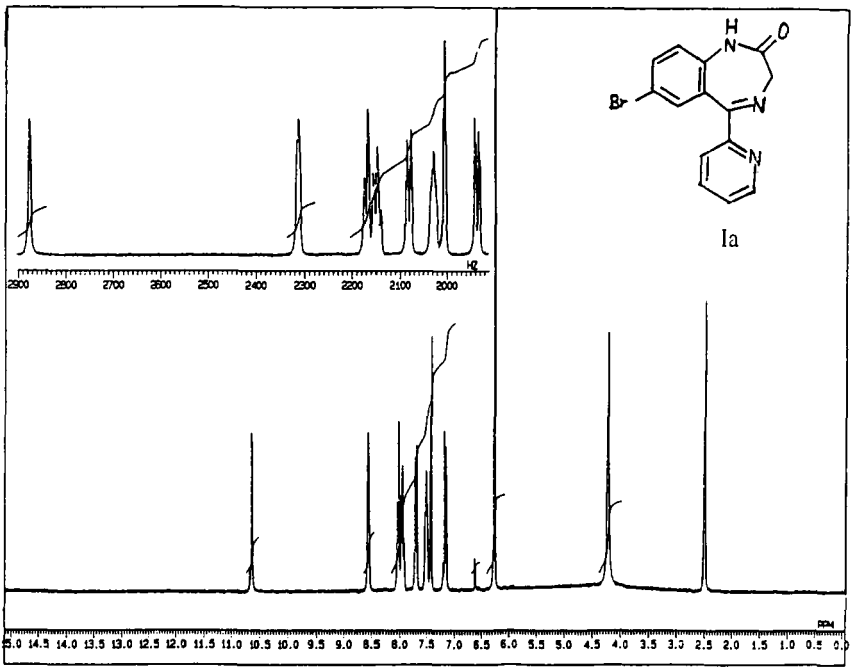


Fig.1 The ^{1}HNMR spectrum of 20 mg intact bromazepam (I_a) in DMSO - d_6 containing 8 mg maleic acid.

The importance of the described ^{1}HNMR technique arises from the finding that, the sharp singlet at 4.2 ppm of the intact forms of both drugs is absent in the spectra of their degradates; benzophenone derivatives I_b and II_b (figs. 2 and 4).

In addition, the resonance peak of the amide's hydrogen of each drug (near 10.7 - 10.8 ppm) disappeared due to its conversion to the free, more basic primary amine - hydrogen which undergoes more rapid proton exchange[18]. Moreover, it has been found that glycine which is incompletely soluble in DMSO - d_6 , has a singlet corresponding to two methylene protons appearing at 3.7 ppm , fig. 5, which is quite apart from the peak of interest at 4.2 ppm. These conditions allow quantitative determination of both drugs in

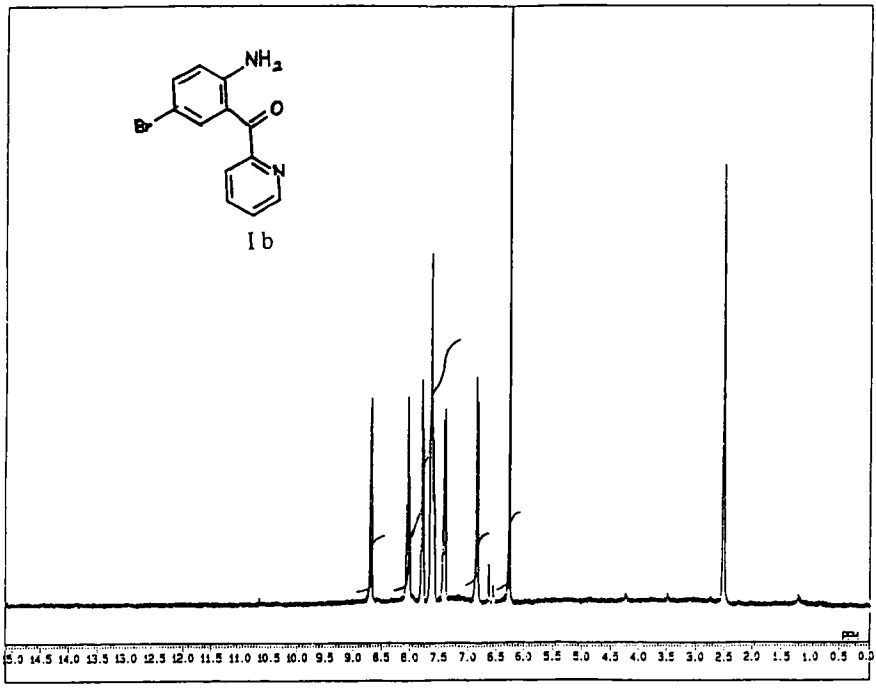


Fig.2 The ^{1}HNMR spectrum of 20 mg degraded bromazepam (I_b) in DMSO - d_6 containing 8 mg maleic acid.

presence of their degradation products even without prior extraction .

Although it has been reported that delorazepam II_a can be degraded at 100 ^oC by reflux with 6 N HCl for 1 hr. [1,4] , yet degradation was found to be incomplete even when using longer time (up to 2.5 hrs) as revealed by TLC[4] and ^{1}HNMR techniques. However, several trials were carried out to obtain complete degradation of II_a, which has been achieved by refluxing with 6 N HCl at 150 ^oC for 1.25 hrs. This resistance of II_a may be due to the presence of the electron - withdrawing chloride in the 2'- phenyl ring which stabilizes the molecule towards hydrolytic attack by hydrogen bonding with the protonted azomethine group[19].

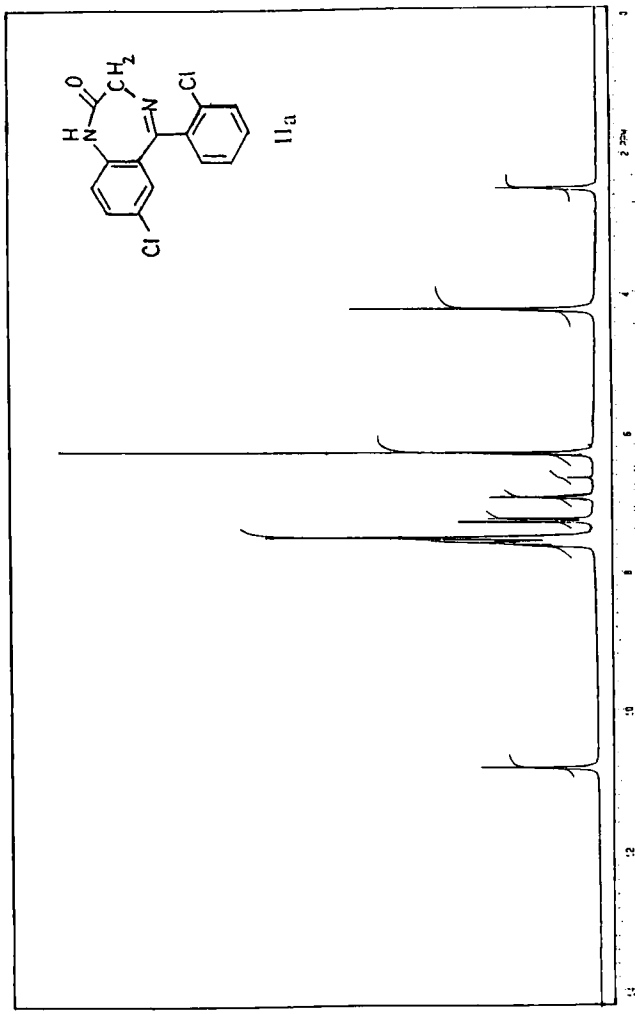


Fig.3 The ^{1}HNMR spectrum of 15 mg intact delorazepam (II_a) in DMSO - d_6 containing 8 mg maleic acid.

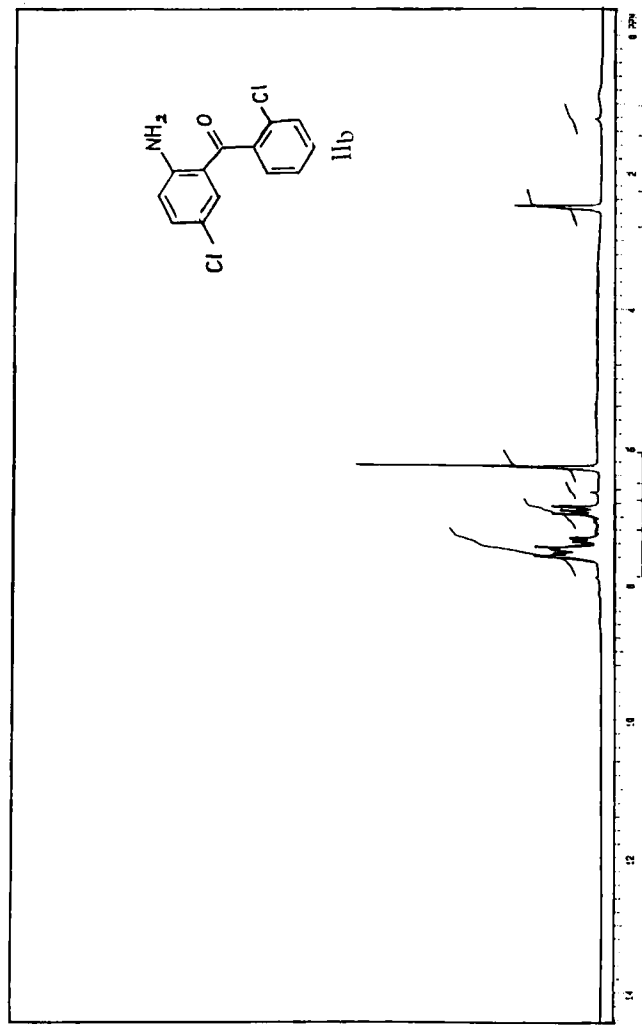


Fig.4 The ^{1}HNMR spectrum of 15 mg degraded delorazepam (II_b) in DMSO d_6 containing 8 mg maleic acid.

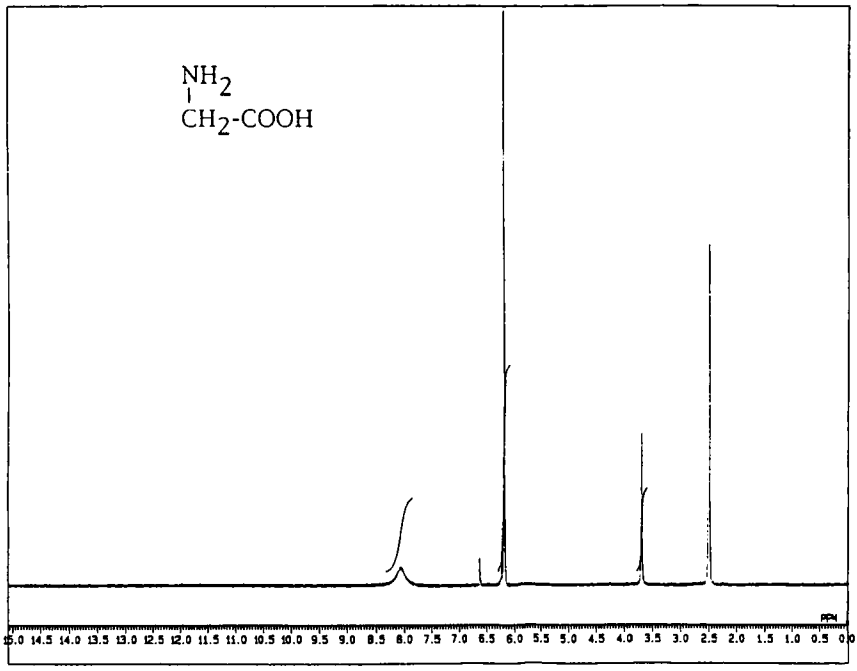


Fig.5 The ^{1}HNMR spectrum of 5 mg glycine in DMSO - d_6 containing 8 mg maleic acid.

It has to be mentioned that the solvent - impurity peak that may interfere with the peak of interest at 4.2 ppm, was overcome by the addition of very few drops of trifluoroacetic acid.

Pure samples of each of the two drugs (5-25 mg)were analysed by the proposed ^{1}HNMR method with mean accuracies of 99.7±0.92 % and 100.5 ± 0.79 % for I_a and II_a , respectively : table(1).

Table (2) summarizes the results obtained by the analysis of laboratory prepared mixtures of the intact and degraded drugs by the proposed ^{1}HNMR method, in addition to the B.P.(1993) [12] and the manufacturer's [16] procedures for I_a and II_a , respectively. The latter procedure recommends direct absorbance

Table(1) **Determination of Pure Samples of Bromazepam and Delorazepam by the Proposed ^{1}HNMR Method.**

Bromazepam				Delorazepam			
Taken mg. ml^{-1}	Integral Ratio*	Found mg. ml^{-1}	accuracy %	Taken mg. ml^{-1}	Integral Ratio*	Found mg. ml^{-1}	accuracy %
5 .0	0.225	4 .905	98 .10	5 .0	0.240	5 .050	101.00
11.0	0.507	11.053	100.48	8 .5	0.401	8 .437	99 .26
14.0	0.644	14.039	100.28	10.5	0.501	10.541	100.39
18.5	0.851	18.574	100.40	15.0	0.718	15.107	100.71
20.0	0.921	20.078	100.39	19.0	0.914	19.231	101.22
23.4	1.061	23.130	98 .85	21.1	1.017	21.398	101.41
25.0	1.140	24.852	99 .41	25.0	1.185	24.932	99 .73
Meam ±C.V.			99.7±0.94%				100.5±0.79%

* Average of three measurements.

Table (2) **Determination of Intact Bromazepam and Delorazepam in Mixtures of Intact and Degraded Drugs by ^{1}HNMR, B.P. (1993)[12] and Manufacturer's[16] Methods.**

Sample No.	Bromazepam				Delorazepam			
	^{1}HNMR		B.P . (1993) [12]		^{1}HNMR		Manufacture's[16]	
	Content of degraded drug * %	Recovery of intact drug %	Content of degraded drug * %	Recovery of intact drug %	Content of degraded drug * %	Recovery of intact drug %	Content of degraded drug * %	Recovery of intact drug %
1 .	20	100.71	20	119.77	20	99 .73	20	106.71
2 .	38	99 .70	30	139.13	30	101.23	30	109.05
3 .	45	101.33	40	159.15	40	98 .89	40	115.08
4 .	56	99 .52	50	197.31	50	100.99	50	123.81
5 .	60	101.21	60	243.47	60	100.19	60	131.67
6 .	71	100.79	70	345.32	75	100.62	70	142.86
7 .	80	101.90			80	101.42	80	171.43
Meam ± C.V.		100.7 ± 0.86%				100.4 ± 0.90%		

* Of total weight

Table (3) **Determination of Bromazepam and Delorazepam in Their Tables by the Proposed ^{1}HNMR Method.**

Preparation	Claimed (mg)	Found (mg)	Recovery %
Calmepam Tablets (1.5 mg) B.N. 410635	5.0	4.650	93.00
	10.0	9.425	94.25
	20.0	18.938	94.69
Mean ± C. V.			94.0 ± 0.93 %
Calmepam Tablets (3 mg) B.N. 300	13.5	13.527	100.20
	21.0	20.821	99.15
	25.0	25.223	100.89
Mean ± C. V.			100.1 ± 0.88 %
E. N. Tablets (0.5 mg) B.N. 410612	7.5	7.233	96.44
	14.0	13.360	95.43
	20.0	19.378	96.89
	25.0	23.965	95.86
Mean ± C. V.			96.2 ± 0.67 %

Table (4) **Statistical Analysis of the Results Obtained by the Proposed ^{1}HNMR , B.P. (1993)[12] and Manfacturer's[16] Methods.**

	Bromazepam		Delorazepam	
	^{1}HNMR Method	B. P. (1993) Method[12]	^{1}H NMR Method	Manufacturer's Method[16]
Concentration range	5 - 25 mg. ml^{-1}	250 mg.	5 - 25 mg. ml^{-1}	10 - 30 ug. ml^{-1}
Mean	99.68	99.98	100.50	100.05
±S.D.	0.46	0.92	0.79	0 .97
n	7	6	7	6
Variance	0.212	0.846	0.624	0.941
$F_{(5.0)}$	3.99	———	0.633	———
$t_{(1.796)}$	0.892	———	1.637	———

* Figures in parenthes are the corresponding theoretical values of F and t (P= 0.05).

measurements of II_a solution in 0.5 N HCL at 287 nm, making use of its (A 1 % , 1cm) equal to 320 . It is obvious from this table that good recoveries of the intact drugs were obtained by the ^{1}HNMR method in presence of up to 80% of their degradates whereas much higher results were obtained for both drugs using B.P. (1993) [12] and the manufcturer's methods [16]. Therefore, the ^{1}HNMR method is proved to be more selective and stability - indicating for the two drugs, incontrast to the other two mentioned non - differentiating methods.

On applying the proposed method for the determination of I_a in calmepam tablets and II_a in E. N. tablets, resonably accurate and precise results were obtained ; table (3) . However, the tablets' powders had to be extracted firstly with chloroform, evaporated to dryness and the residue determined as a pure sample , due to the very high content of additives in both tablets relative to the low active drug concentrations. Many organic solvents were tried for such extraction but chloroform was found to be most efficient.

Statistical analysis of the results obtained by the ^{1}HNMR, B. P. (1993)[12] and manufacturer's methods[16] revealed no significant difference, within a probability of 95% of being correct; table (4).

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