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STABILITY STUDY OF LORAZEPAM IN SOLID DOSAGE FORM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A reverse phase column with MeOH-H_2O as mobile phase and detection at 230 nm was employed for the determination of lorazepam and degradation products in tablet formulation. The mean coeficient variation (n=6) for the entire analytical method was 1.15%. A working calibration curve over a concentration range of 5 to 250 ng of lorazepam was obtained and the recovery (n=3) was 100.5%. Limits of detection varied from 1.6 to 3.2 ng according to the compounds. Natural and thermal stability of the drug and tablets were carried out since the method was suitable for stability indicating studies. A comparative TLC method was also performed. The effect of the type and concentration of acid and the content of methanol in reaction medium of hydrolysis of lorazepam were also investigated. Degradation products were characterized by HPLC and TLC by comparing them to authentic samples. The first

degradation product that appeared was the quinazoline-carboxaldehide and 2-amino-2',5-dichlorobenzophenone was not detected. The additives in tablets decreased drug stability and degradation pathway followed by the tablets was similar to the drug under thermal conditions.

INTRODUCTION

1,4-benzodiazepines are psychotherapeutic drugs characterized by sedative, hypnotic and anti-convulsivant properties.

Many analytical methods have been proposed for the determination of benzodiazepines, their metabolites in biological fluids and degradation products in pharmaceutical dosage forms such as spectrophotometry, thin layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance (NMR) and high performance liquid chromatography (HPLC). This latter methodology plays a dominant role, specially for the analysis of thermally labile benzodiazepines as lorazepam[1-7].

Development stability studies of the drug itself and in its pharmaceutical formulations allows a better knowledge of its physico- chemical, pharmacotherapeutic and toxicological behaviour. Therefore, this paper reports the results of an investigation about the stability of bulk lorazepam and in tablet formulation where its degradation products are analized by HPLC.

MATERIALS AND METHODS

HPLC Analysis: Methanol HPLC grade (E. Merck, Darmstadt, F. R. G.) and deionized, glass- bidistilled water solvents were filtered through a 0.45 μm membrane and degassed before used.

Lorazepam (I), 6-chloro-4-(2-chlorophenyl)-2-quinazolinecarboxaldehyde (II), N-[threo-1,2-bis[6-chloro-4-(2-chlorophenyl)-2-quinazolinyl]-2-hydroxyethyl]-6-chloro-4-(2-chlorophenyl)-2-quinazolinecarboxamide (IV) and 6-chloro-4-(2-chlorophenyl)-2-quinazolinecarboxilic acid (VI) were supplied by Wyeth Laboratories (Argentine). 6-chloro-4-(2-chlorophenyl)-2-quinazoline alcohol[8] (III), 6-chloro-4-(2-chlorophenyl)-2 (1H)- quinazolinone[9] (V) and 2-amino-2',5-dichlorobenzophenone [10] (VIII) were obtained according to literature procedures. The (III), (V) and (VII) compounds were purified by TLC and identified by IR, ^{1}H-NMR and mass spectrometry.

The solutions of reference standards (10 µg/ml) were prepared by dilution with mobile phase from a stock solution in methanol (1 mg/ml). A working standard lorazepam solution (12 µg/ml) was used. The lorazepam tablets were provided by Wyeth Laboratories (Argentine).

The HPLC was performed making use of a liquid chromatograph Varian Model 5020 (Palo Alto, C A,U.S.A.). A Micropack MCH- 10 column (300 x 4 mm I. D.) was employed. The mobile phase consisted of methanol- water (70:30). The flow rate was 1.2 ml/min and the temperature was 32°C. The injection volume was 10 µl. The detection was performed at 230 nm and 0.05 a.u.f.s.

The sample preparation was obtained by grinding twenty tablets to a fine powder and an accurate amount of its equivalent to 3 mg of lorazepam was transfered to a stoppered flask and 25 ml of methanol accurately measured were added. The mixture was sonicated for ten minutes and centrifuged. An aliquot of the supernatant solution was diluted 1:10 with mobile phase. It was filtered through a 0.45 µm menbrane before the injection.

Semiquantitative TLC Chromatographic Analysis: TLC was performed on glass- plates silica 60 F_{254} (20 x 20 cm, Merck, Darmstadt, F. R. G.). The solvents used were of chromatographic grade (Merck). The plates were developed in the mobile phase chloroform- toluene-methanol (52:48:7).

For the sample preparation, powdered tablets were extacted first with chloroform and then with methanol and the extracts were mixtured. Aliquots containing 120 µg of lorazepam were spotted.

RESULTS AND DISCUSSION

In the evaluation of lorazepam by the HPLC method proposed, there is not interference of additives and degradation products. These latter may be also determined.

A calibration curve over the range 5- 250 ng for lorazepam and degradation products were obtained. The detection limits varied from 1.6 to 3.2 ng according to the compound studied. The quantitation was carried out by the external standard method. The reproducibility system, for six injections of a lorazepam reference standard solution, was 0.62%. The reproducibility of the analytical method was 1.15% for n=5 of replicated injections. The mean recovery (n=3) of lorazepam was 100.5% for tablet formulation.

The stability- indicating studies were performed by HPLC and TLC for bulk drug and tablets under thermal conditions at 37°C and 60°C in air and in 80% R. H. during six months (Figure 1) and at room temperature for one year.

For lorazepam itself, a minimal degradation was observed at 37°C and only quinazolinecarboxaldehyde (II) (1%) was detected by TLC and at 60°C quinazoline alcohol (III), compound IV and an unknown spot at R_f

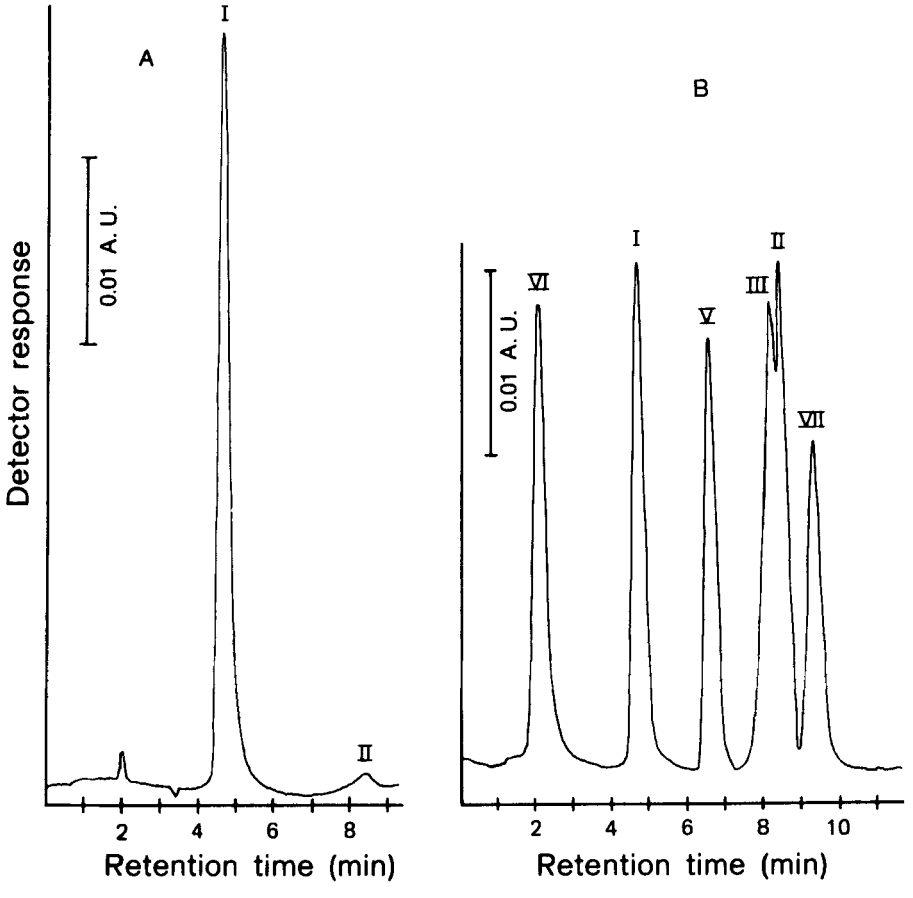

FIGURE 1

A. Chromatogram of tablets under thermal conditions (37°C in air during three months). B. Chromatogram of a reference standard solution of lorazepam and degradation products in operating conditions.

0.44 were also detected. Further quinazolinone (V) and quinazolinecarboxilic acid (VI) were formed in a greater degradation state (Table 1). The quinazolinone (V) has been reported as a metabolite[11,12].

Under acidic conditions, hydrolysis of lorazepam is dependent on the kind and concentration of acids employed, in the presence or not of alcohols, so

TABLE 1
Retention Time and R_f Values for Lorazepam and Degradation Products.

Compound	Retention Time (min)	R_f
VI	2.1	0.05
I	4.6	0.15
V	6.7	0.24[a]
III	8.1	0.51
IV	b	0.54
II	8.4	0.60
VII	9.3	0.82

[a] blue fluorescence at long wavelength
[b] low solubility in mobile phase

degradation products obtained varied in quality and quantity. However, 2-amino-2',5-dichlorobenzophenone (VII) was obtained in 4N HCl medium but not in 4,5N AcH and only quinazolinecarboxaldehyde (II) was the main product in the latter condition. In the oxidative degradation 2-amino-2',5-dichlorobenzophenone (VII) was obtained neither.

Natural and acelerated degradation of lorazepam in the solid dosage form was similar to that observed for the drug at 60°C. The quinazolinecarboxaldehyde (II) was the first product formed but its amount did not increase according to degradation which confirmed its further transformation in others products. The benzophenone (VII) was absent in tablets (Tables 2 and 3). A major degradation is produced at 60°C where quinazolinecarboxilic acid (VI) and quinazolinone (V) were determined.

At room temperature for one year a 97.7 % of lorazepam was recovered and 0.8 % of quinazolinecarboxaldehyde (II) was determined by HPLC. The same compounds formed at 37°C were detected by TLC.

TABLE 2

Determination of Lorazepam in Tablets at 60°C[a]

Time	Degradation Condition	mg/Tablet	I (%)	II (%)	V (%)	VI (%)
initial		1.005	100.5	-	-	-
3 months	in air	0.707	70.3	1.9	-	0.8
	80 % R.H.	0.340	33.8	2.0	0.5	2.1
6 months	in air	0.650	64.7	2.3	0.6	8.9
	80 % R.H.	0.052	5.2	2.2	1.6	19.7

[a]mean values of replicated injections of three samples.

TABLE 3
Determination of Lorazepam in Tablets at 37°C[a]

Time	Degradation Condition	mg/Tablet	I (%)	II (%)
initial		1.005	100.5	-
3 months	in air	0.985	98.0	1.2
	80 % R.H.	0.978	97.3	1.3
6 months	in air	0.947	94.2	1.2
	80 % R.H.	0.941	93.6	1.1

[a]mean values of replicated injections of three samples.

CONCLUSIONS

This accurate and precise methodology developed by HPLC allows the determination of lorazepam in presence of its degradation products and is useful and suitable for routinery assays and for stability testing of lorazepam in solid dosage forms. The results obtained suggested that the additives in tablets decrease drug stability.

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