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Short Communication

Stability study of ethyl loflazepate in bulk drug, solution and dosage form by liquid chromatography

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Introduction

The benzodiazepines are an important group of psychotherapeutic drugs used as sedative hypnotic and anticonvulsivant agents. Several analytical methods have been described in the literature for the identification and determination of benzodiazepines in different matrices such as TLC, GC and HPLC. Sometimes, TLC [1] lacks sensitivity and selectivity for many requirements in pharmaceutical analysis, and, in most cases GC [2, 3] requires derivatization and this methodology is not suitable for thermolabile benzodiazepines such as chlordiazepoxide and oxazepam. LC appears to be the technique of choice and numerous methods using it have been published [4–7]. Ethyl loflazepate (Fig. 1) is an active anxiolytic drug and its biotransformation has been investigated previously [8, 9]. LC methods for the analysis in biological samples have been described [10, 11].

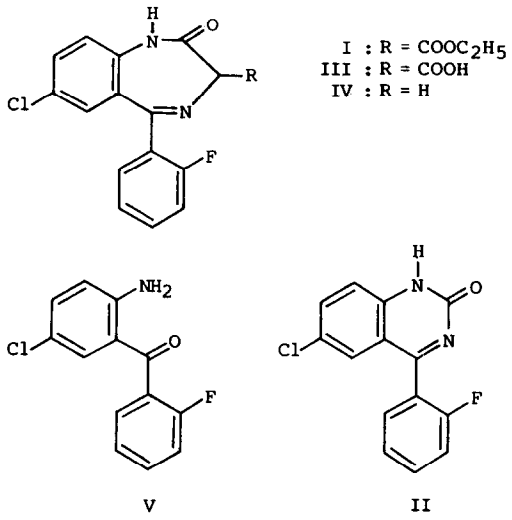

Figure 1
Structures of ethyl loflazepate and degradation products.

Experimental

Materials

All chemicals used were of analytical grade. Acetonitrile and water were HPLC grade. The solvents were filtered through a 0.45 μm membrane and degassed.

Ethyl loflazepate (I) and 7-chloro-5-(2'-fluorophenyl)-2-oxo-2,3-dihydro (1*H*)-3-carboxilic-1,4-benzodiazepine acid (III) were kindly supplied by Sanofi Recherche (Montpellier, France). 7-chloro-5-(2'-fluorophenyl)-2-oxo-2,3-dihydro (1*H*)-1,4-benzodiazepine (IV); 2-amino-5-chloro-2'-fluorobenzophenone (V) were prepared in house. 6-chloro-4-(2'-fluorophenyl)-2 (1*H*)-quinazolinone (II) was obtained according to a literature procedure [12].

*7-chloro-5-(2'-fluorophenyl)-2-oxo-2,3-dihydro (1*H*)-1,4-benzodiazepine (IV).* Ethyl loflazepate was hydrolysed with 0.25 M sodium hydroxide aqueous solution at a concentration of 1 mg ml^{-1} for 24 h at room

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temperature. Under this condition the sodium salt of III was obtained as the only compound which was easily decarboxylated to IV. Then, the pH was adjusted to 3–4 with acetic acid in an ice water bath to precipitate a yellow solid which was filtrated and washed on filter paper. This residue was recrystallized from methanol–water and identified by mass spectrometry and IR [8].

2-amino-5-chloro-2'-fluoro benzophenone (V). An aqueous solution of 1 mg ml^{-1} of ethyl loflazepate in 0.5 M sodium hydroxide was heated under reflux in a water bath for 3 h. As a result a yellow solid precipitated. The suspension was cooled to room temperature and the solid was separated and washed with water. Then, it was recrystallized from aqueous methanol and identified by mass spectrometry, ^{1}H-NMR and IR.

Liquid chromatography

The chromatographic analyses were performed with a liquid chromatograph Varian Model 5020 (Palo Alto, CA, USA). A Micropack MCH-10 column (300 × 4 mm i.d.) was employed. The mobile phase consisted of acetonitrile–water (50:50, v/v). The flow rate was 1.0 ml min^{-1} and the temperature was 30°C. The injection volume was 10 μl and detection was performed at 230 mm, 0.05 aufs.

Standard solutions for LC

Stock solutions of each compound were prepared in methanol at 1 mg ml^{-1} and found to be stable for at least 1 week when stored at 4°C. Reference standard solutions containing 10 μg ml^{-1} were obtained by dilution of the stock solution in mobile phase and they were freshly prepared every day.

For tablet quantification a working standard solution of ethyl loflazepate containing 16 μg ml^{-1} was obtained by a suitable dilution in mobile phase of the stock solution.

Solutions for pH stability studies

Solutions of 1 mg ml^{-1} of ethyl loflazepate ranging from pH 3.6 to 9.5 were obtained by dissolution with a mixture of methanol–0.05 M phosphate buffer (60:40, v/v). A 0.02 M hydrochloric acid solution was used for pH 2.3. All the aqueous solutions were taken to ionic strength 0.15 with sodium chloride, which corresponds to the strongest obtained for the dibasic sodium phosphate solution employed.

Tablet preparation

Twenty tablets, whose coatings had been gently removed by scratching, were ground to a fine powder in a mortar. An accurately weighed amount equivalent to 2 mg of ethyl loflazepate was transferred to a stoppered flask and 25 ml of methanol were added. The mixture was sonicated for 20 min and centrifuged. An aliquot of the supernatant solution diluted 2:10 with mobile phase was filtered through a 0.45 μm membrane before the injection.

Semiquantitative TLC chromatographic analysis

TLC was performed on glass-plates silica 60 F_{254} (20 × 20 cm, Merck, Darmstadt, Germany). The solvents used were of chromatographic grade. Chloroform–diisopropyl ether–ethanol (70:25:5, v/v) was used as mobile phase [9]. In a preliminary qualitative study, powdered tablets were first extracted with chloroform, then with methanol and the extracts were mixed to assure the extraction of all the possible degradation products. Aliquots containing 120 μg of ethyl loflazepate were spotted.

Results and Discussion

In the LC method proposed for the evaluation of ethyl loflazepate there was no interference of additives or degradation products, which may also be determined. To prepare a calibration curve a series of dilutions from the stock solutions of ethyl loflazepate and degradation products were made to cover a range of 0.5–20 μg ml^{-1}. The linearity of the method was evaluated by triplicate injections of each solution. Under experimental conditions the detection limits ($S/N = 3$) varied from 2.0 to 7.0 ng according to the compound studied. The reproducibility of the chromatographic system for five injections of ethyl loflazepate reference standard solution was 0.80%. The quantification was carried out by the external standard method. The mean recovery ($n = 3$) of ethyl loflazepate content in tablet formulations was 100.3% under experimental conditions.

The stability indicating study was performed by LC and TLC for bulk drug and tablets under thermal condition at 50°C at 80% R.H. for a period of 4 months (Fig. 2, Tables 1 and 2) and at room temperature for 1 year. Minimal

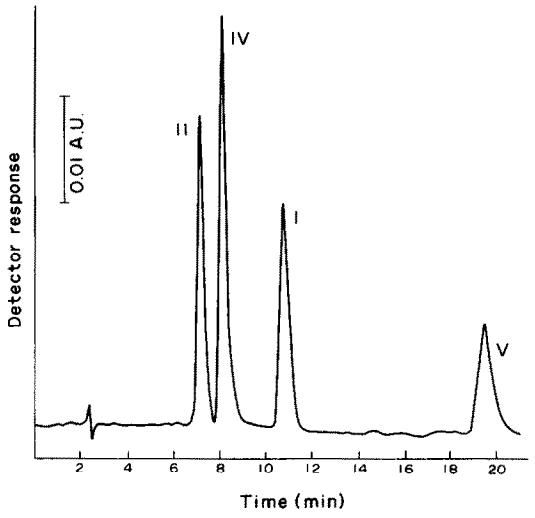

Figure 2
Chromatogram of a reference standard solution of ethyl loflazepate and degradation products in operating conditions.

Table 1
Values of R_f and k' for ethyl loflazepate and degradation products

Compound	k'	R_f
I	3.21	0.62
II	1.80	0.19*
III	†	0.00
IV	2.22	0.37‡
V	6.23	0.90

*Light blue fluorescence at long wavelength.
†Decarboxylated by the mobile phase.
‡Yellow fluorescence at long wavelength.

Table 2
Determination of ethyl loflazepate in tablets at 50°C in 80% R.H.*

Time (months)	Milligrams per tablet	Percentage
Initial	2.06	103.0
1	1.98	99.0
2	1.93	96.5
3	1.92	96.0
4	1.91	95.5

*Mean values of replicated injections of three samples; RSD: 1.04% (n = 5).

degradation of ethyl loflazepate drug was observed at 50°C at 80% R.H. during 4 months and only quinazolinone (II) was detected (<0.1%) by TLC. This compound has been reported previously as a metabolite [9].

In the accelerated degradation of ethyl loflazepate in the solid dosage form, the quinazolinone (II) was the first product found. Then an unknown compound at R_f 0.45 appeared after further degradation. The benzophenone (V) appeared neither in these tablets nor in those used in previous accelerated degradation studies of lorazepam and oxazepam [7, 13].

The tablets kept at room temperature for 1 year showed a similar degradation to that observed in the drug at 50°C. Ninety-six per cent of the initial ethyl loflazepate was determined by LC.

Under acidic conditions hydrolysis of ethyl loflazepate was dependent on the kind and concentration of acids employed and was not influenced by the presence of alcohols. The same behaviour was observed with lorazepam [7]. Under the experimental conditions at pH 2.30, compound IV was the principal degradation product formed. The same results were observed when weak acid solutions were employed, such as acetic acid 25% in aqueous solution, while benzophenone (V) appeared in 1 N hydrochloric acid methanolic aqueous solution. Between pH 3.6 and 6.2 the unknown compound at R_f 0.45 was mainly formed but at pH 6.2 compound IV and quinazolinone began to appear. From pH 7.3 to 9.4 the major product was IV whose proportion increased with pH and hydrolysis time while the quantity of quinazolinone (II) remains constant.

The ethyl loflazepate solutions of various pH values decomposed according to pseudo first-order law at room temperature (Fig. 3). From the pH rate profile curve (Fig. 4) the optimum

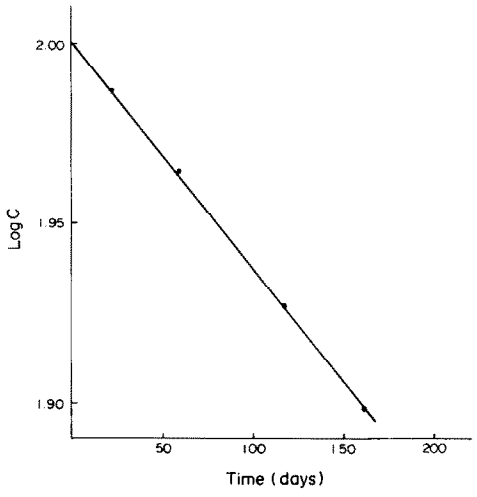

Figure 3
Pseudo first-order plot of ethyl loflazepate decomposition at pH 6.20.

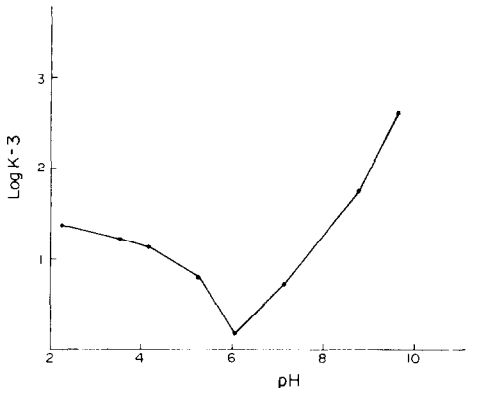

Figure 4
Profile curve of pH–rate for ethyl loflazepate.

apparent pH for the stability of ethyl loflazepate appeared to be 6.20. Alkaline pH accelerates degradation faster than acidic pH. The results obtained show the importance of a knowledge of the acid–base properties of the excipients in order to obtain a stable formulation of the drug.

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