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Short Communication

High-performance liquid chromatographic determination of clobazam and one of its pharmaceutical formulations

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Introduction

Clobazam [7-chloro-1-methyl-5-phenyl-1H-1,5-benzodiazepine-2,4-(3H,5H)-dione] is a 1,5-benzodiazepine that has demonstrated anti-anxiety and anti-epileptic properties [1–4]. Several methods have been reported for the quantitative analysis of clobazam in biological fluids. These methods include a fluorimetric method [5], a radioreceptor technique [6], gas–liquid chromatography [7–10], and high-performance liquid chromatography (HPLC) [11].

This paper describes a simple, precise and direct high-performance liquid chromatographic method for the determination of clobazam as the bulk drug and one of its formulations (Frisium 10).

Experimental

Instrumentation

The apparatus used was a Varian Model 5000 liquid chromatograph, equipped with a variable wavelength Model UV50 detector and a Model 9176 recorder. Separation was performed on a Micro-pack-Si-10 (30 cm × 3.9 mm i.d.) column. Analytical samples were introduced onto the column by means of a 10 μl loop valve (Rheodyne 7125).

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HPLC conditions

The mobile phase consisted of concentrated ammonium hydroxide (28–30%, v/v) in methanol (0.75:99.25, v/v). The operating conditions were: ambient temperature (about 21°C); flow rate 2 ml/min, detector wavelength 254 nm; absorbance range 0.05 a.u.f.s.

Reagents

Authentic clobazam tablets (Frisium 10 mg tablets, batch No. 25/L058) were obtained from Hoechst Aktiengesellschaft (Frankfurt am Main, West Germany), quinine base from Merck Laboratories (Darmstadt, West Germany), and methanol (spectroscopic grade) from Fluka AG (Buchs, Switzerland). Ammonia solution was analytical grade obtained from BDH (Poole, Great Britain).

Methods

Assay of authentic clobazam and clobazam tablets by HPLC. A stock solution of clobazam in methanol (50 μg/ml) was used to prepare five calibration solutions in the concentration range 0.5–2.5 μg/ml, containing quinine (4 μg/ml) as internal standard, with the mobile phase as diluent.

For clobazam tablets, twenty tablets were accurately weighed and powdered. An accurate weight equivalent to 5 mg of clobazam was transferred into a 100 ml volumetric flask. The active ingredient in the powdered tablet was extracted with 90 ml methanol by vigorous shaking for 15 min. The volume was then adjusted to 100 ml and the solution filtered through a Durapore filter (Waters Associates, Bedford, MA), one which is compatible with common HPLC solvents both aqueous and organic. A 3 ml aliquot of the filtrate was diluted to 100 ml with the mobile phase containing quinine (4 μg/ml) as the internal standard to give a concentration of 1.5 μg/ml clobazam.

Triplicate 10 μl injections of each of the five clobazam calibration solutions were made. The ratio of clobazam peak area to the internal standard peak area was then plotted versus the concentration of clobazam.

Triplicate 10 μl injections of the sample solution (clobazam in tablets) were also made and the average peak area ratio was determined. The concentration of clobazam in the sample was then found by graphical interpolation. Similarly, spiked samples were prepared and the total amount of clobazam determined.

Assay of clobazam by ultraviolet spectrophotometry. The spectroscopic method cited in the *British Pharmacopoeia* [12] for the assay of diazepam tablets was adopted for the determination of clobazam (no official method of assay for clobazam is available).

A 0.1% m/v stock solution of clobazam in 0.5% w/v sulphuric acid–methanol was prepared. Dilutions of 2, 4, 6, 8 and 10 ml to 100 ml, respectively, were made in the sulphuric acid–methanol solution to give concentrations of 0.002–0.01% m/v clobazam. Absorbances were measured against the reagent in a 1 cm cell at the maximum absorption wavelength of 290 nm.

For clobazam tablets, an accurate weight equivalent to one half tablet was shaken with about 90 ml of the acidic methanol solution for 15 min, and then made up to volume with the same solution. After filtration, the absorbance was measured at 290 nm and the concentration of clobazam was determined from the calibration graph.

The above-mentioned procedure for the assay of clobazam by UV spectrophotometry was repeated by extraction with methanol only (in the absence of sulphuric acid).

Results and Discussion

A typical chromatogram for a clobazam sample (in its tablet dosage form) is shown in Fig. 1. Peaks 1 and 2 correspond to clobazam and the internal standard, respectively. Their respective retention times are 2 and 3 min, at a flow rate of 2 ml/min. A plot of peak area ratios versus concentration was linear ($y = 0.0118 + 0.3372x$) over the concentration range 0.5–2.5 μg/ml, with a correlation coefficient (r) value of 0.999. The relative standard deviation of the method was found to be 2.41% for ten replicates of 1.5 μg/ml, indicating high reproducibility. Although the mobile phase was alkaline, the high methanol concentration appears to protect the silica from degradation and no significant loss of separation performance was noticed over several months.

The mean recovery of clobazam from its tablet dosage form (Frisium 10) was 98.1 ± 0.8%, and the mean recovery of five replicates of clobazam (0.5 μg/ml) added to the previously assayed dosage (1 μg/ml) containing the active ingredient was 99.8 ± 1.5%.

The UV method was used to validate the results obtained using the HPLC method. Methanol–sulphuric acid was used to comply with the official pharmacopoecial method for the assay of diazepam adopted here for the determination of clobazam, although similar results were obtained by extraction with methanol alone.

Beer's law was obeyed ($A = 0.0079 + 0.0076c$) over the concentration range 20–100 μg/ml. The recovery of clobazam from its tablet dosage form was 99.5 ± 0.25%.

In the method reported by Brachet-Lirmain *et al.* [11], the calibration curve for clobazam was linear between 50 and 500 ng/ml. Therefore, the sensitivity of the present HPLC method (linearity range 0.5–2.5 μg/ml) is comparable to that reported by

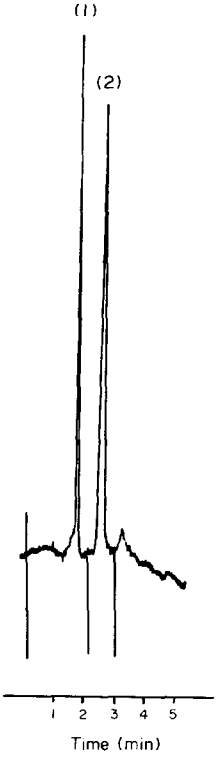

Figure 1
Chromatogram of clobazam (**1**) and internal standard (**2**). Separation on a Micro-pack Si-10 (30 cm × 3.9 mm i.d.) column with methanol–aqueous ammonia (99.25:0.75, v/v) eluent and UV detection at 254 nm.

Brachet-Lirmain *et al.* [11], provided that they injected 100 μl compared to the 10 μl volume injected in the current work, making the on-column volumes 5–50 ng and 5–25 ng, respectively.

The HPLC procedure reported in this communication offers a simple, precise and direct method for the determination of clobazam in its tablet dosage form.

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