Journal of Chromatography, 368 (1986) 180–183
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 919

Note

Determination of temazepam and its major degradation products in soft gelatin capsules by isocratic reversed-phase high-performance liquid chromatography

SALLY M. GORDON*
School of Pharmacy, Portsmouth Polytechnic, King Henry I Street, Portsmouth, PO1 2DZ (U.K.)
and
LESLEY K. FREESTON and ANTONY J. COLLINS
Wessex Regional Pharmaceutical Quality Control Laboratory, Queen Alexandra Hospital, Cosham, Portsmouth, PO6 3LY (U.K.)
(First received May 12th, 1986; revised manuscript received July 10th, 1986)

Temazepam (3-hydroxydiazepam; 7-chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one) is used extensively as a hypnotic for the relief of insomnia[1]. It is marketed by a number of pharmaceutical companies in the United Kingdom, and is administered usually as soft gelatin capsules containing either 10 mg or 20 mg of temazepam in a non-aqueous solution.

Most of the published methods for the detection and quantification of temazepam have been developed for the examination of biological samples, such as urine and plasma[2,3]. The techniques are often screening systems, allowing any of a number of benzodiazepine derivatives to be identified, as in, for example, the gas chromatographic method of Douse[4] and the thin-layer chromatographic (TLC) method of Bakavoli *et al.*[5]. Cotler *et al.*[6] developed a rapid, sensitive and specific high-performance liquid chromatographic (HPLC) assay for diazepam and its major metabolites (temazepam, oxazepam and nordiazepam) in plasma, blood and urine samples from humans and cats. This method used a C_{18}-type reversed-phase column and methanol–water as the mobile phase, and enabled the plasma concentration–time profiles of diazepam and nordiazepam in humans and cats to be monitored. The problems of sample purification and of low drug concentrations encountered in the analysis of biological materials do not arise during the examination of pharmaceutical preparations. Also, the degradation products likely to be present in the capsules are not necessarily the same compounds as the metabolites of temazepam found in urine and plasma. Thus, the previously reported methods are not suitable for the examination of capsules.

At present, neither temazepam nor temazepam preparations are the subjects of monographs in the British Pharmacopoeia, and information regarding assay methods and limits for impurities in the capsules is scarce. At least one British manufacturer uses an ultraviolet spectrophotometric assay for temazepam, together with a TLC method for the detection and estimation of its major breakdown products, diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one)

0021-9673/86/$03.50

and 7-chloro-1-methyl-5-phenyl-4,5-dihydro-2H-1,4-benzodiazepin-2,3-9-(H)-dione (I), in soft gelatin capsules. Such methods are time-consuming, and TLC does not allow the amounts of diazepam and compound I to be accurately measured. Thus, an HPLC technique has been developed which allows the assay of temazepam and the estimation of degradation product levels to be performed simultaneously on individual capsules.

EXPERIMENTAL

The chromatographic system consisted of an Applied Chromatography Systems 750-03 pump, a Rheodyne 7125 injection valve fitted with a 20-μl sample loop, and either a Pye Unicam PU 4025 variable-wavelength absorbance detector operated at 258 nm, linked to a Philips PM 8251A recorder (chart speed 300 mm/h, operating voltage 10 mV), or a Hewlett-Packard HP 1040A linear photodiode array detector. The assay was carried out using a 250 mm $\times$ 4.6 mm I.D. stainless-steel column packed with LiChrosorb 10 RP-18 (Chrompack, London, U.K.), and the mobile phase was methanol–water (60:40). A flow-rate of 3.5 ml/min was found to give adequate separation of temazepam from its breakdown products, while still allowing assays to be performed rapidly. The methanol used was of chromatographic grade (Fisons, Loughborough, U.K.).

Standard solutions were prepared by dissolving temazepam (R.P. Scherer, Swindon, U.K.) in methanol. Test solutions were made by cutting the soft gelatin capsules open, and rinsing their contents into volumetric flasks using methanol. The volumes of the test solutions were adjusted to give final nominal concentrations of 0.1 mg/ml. The diazepam solution was prepared by dispersing a "Valium" tablet (2 mg diazepam/tablet; Roche Products, Welwyn Garden City, U.K.) in methanol using an ultrasonic bath, making the volume up to 20 ml with methanol, filtering through a 0.45-μm membrane filter (Acrodisc CR; Gelman Sciences, MI, U.S.A.), and diluting 5 ml of the filtrate to 100 ml with methanol, to give a final concentration of 0.005 mg/ml diazepam. A solution containing 0.005 mg/ml of compound I [Generics (U.K.), Potters Bar, U.K.] in methanol was prepared also. All solutions were filtered through 0.45-μm membrane filters before use. Injections were made using the loop-filling technique.

RESULTS AND DISCUSSION

The retention times for temazepam, diazepam and compound I were found to be approximately 4.5 min, 8 min and 2.8 min, respectively. This degree of separation was adequate for the purposes of the present work. Using the PU 4025 detector at 258 nm with a sensitivity of 0.08 a.u.f.s., the height of the temazepam peak was found to be linearly related to the amount of temazepam injected over the range 0.4 to 3 μg (r = 0.9975). Ten consecutive injections of 2 μg temazepam gave a mean peak height of 184.2 mm, and the relative standard deviation (n = 10) was 0.9%. The minimum detectable quantity of temazepam was estimated to be 0.2 μg at a sensitivity of 0.08 a.u.f.s. Using this setting, injections of 20 μl of 0.005 mg/ml solutions of diazepam and compound I gave readily detectable peaks (Fig. 1). However, the difficulties encountered in measuring such small peaks lead to high relative standard

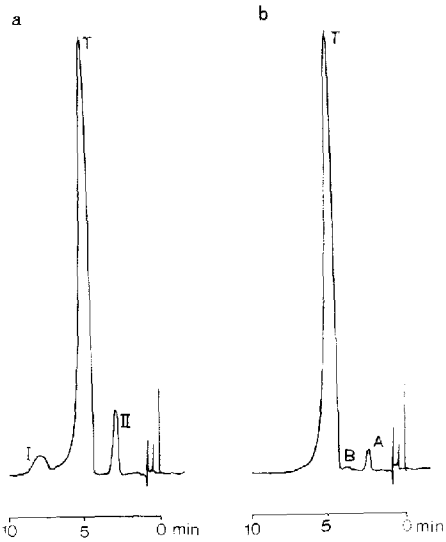

Fig. 1. Chromatograms obtained following injections of: (a) 20 μl of a standard solution containing 0.1 mg/ml temazepam, 0.005 mg/ml diazepam and 0.005 mg/ml compound I in methanol; (b) 20 μl of a solution prepared by dissolving the contents of one 10-mg temazepam capsule [Generics (U.K.), batch 1814133] in methanol, nominal temazepam concentration 0.1 mg/ml. All conditions as described in text, using a Pye Unicam PU 4025 absorbance detector, operated at 258 nm and 0.08 a.u.f.s. T = 2 μg temazepam, retention time 4.6 min; I = 0.1 μg diazepam, retention time 7.8 min; II = 0.1 μg 7-chloro-1-methyl-5-phenyl-4,5-dihydro-2H-1,4-benzodiazepin-2,3-9-(H)-dione (compound I), retention time 2.8 min; A = unknown compound, retention time 2.4 min; B = unknown compound, retention time 3.8 min.

deviations [diazepam, 6.7 mm ± 6.7% (n = 5); compound I, 16.3 mm ± 2.7% (n = 5]. Thus, if the amount of either of these compounds is to be measured, the precision of the technique must be improved by increasing the sensitivity of the detector, and, perhaps, by monitoring peak area, rather than peak height.

The amounts of the breakdown products in these injections (0.1 μg) represent levels of 5% of either diazepam or compound I in a solution nominally containing 0.1 mg/ml temazepam (for instance, one 10-mg capsule to 100 ml solution), which are comparable to the limits in the TLC method used presently. Increasing the sensitivity of the detector allows levels of 1% of either diazepam or compound I to be estimated. Use of the HP 1040A detector will allow detection of even smaller quantities of the breakdown products.

The purity of the temazepam peaks produced by both test and standard solutions was assessed by comparisons of the absorbances at 210 nm and 258 nm ("ratio plots") across the peak (from 3.5 min until 5.5 min after injection) using the photodiode array detector. No co-eluting compounds were detected in either the test or standard solutions. Some extra peaks were noted in the test solutions (Fig. 1). These were not due to either diazepam or compound I, since they had different retention times, and the spectra of the components causing these peaks (190 nm to 340 nm; HP 1040A detector) were not comparable with those of the benzodiazepines. These extra peaks may be due to either excipients in the non-aqueous capsule fill solution, or to materials extracted from the capsule shell by the methanol used to prepare the test solutions. These other compounds do not interfere with the assay.

Typically, assays of samples taken from batches of temazepam capsules received by the hospital direct from the manufacturer give chromatograms similar to that shown in Fig. 1. The temazepam content has been found to be between 95% and 105% of that stated in most instances, and no traces of the breakdown products have been found, even when the detector sensitivity was increased to 0.01 a.u.f.s. Examination of capsules stored at room temperature for periods of up to one year has revealed no apparent change in their temazepam content, and neither diazepam nor compound I has been detected. The capsules will be re-examined periodically to monitor future changes in their chemical composition.

This work shows that HPLC is a suitable method for the assay of temazepam capsules and for the estimation of breakdown products of this drug in pharmaceutical preparations. For routine assays, it should be possible to use only two or three standard temazepam solutions, since the linearity of response has been established, and the limits set in the Wessex Regional Laboratory are 90% to 110% of the stated amount of temazepam per capsule. The photodiode array detector has proved very useful in establishing peak purity, and in the examination of the extraneous peaks seen in the test solutions.

REFERENCES

1 J. E. F. Reynolds (Editor), *Martindale: The Extra Pharmacopoeia*, The Pharmaceutical Press, London, 28th ed., 1982, p. 817.
2 P. C. Ho, E. J. Triggs, V. Heazlewood and D. W. A. Browne, *Ther. Drug Monit.*, 5 (1983) 303.
3 A. C. Mehta, *Talanta*, 31 (1984) 1.
4 J. M. F. Douse, *J. Chromatogr.*, 301 (1984) 137.
5 M. Bakavoli, V. Navaratnam and N. K. Nair, *J. Chromatogr.*, 299 (1984) 465.
6 S. Cotler, C. V. Puglisi and J. H. Gustafson, *J. Chromatogr.*, 222 (1981) 95.