ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
23 (2000) 819–823

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Determination of zopiclone in tablets by HPLC and UV-spectrophotometry

Beata Paw, Genowefa Misztal *

Department of Medicinal Chemistry, Faculty of Pharmacy, Medical Academy, Chodzki Str. 6, 20-093 Lublin, Poland

Received 2 December 1999; received in revised form 18 March 2000; accepted 1 April 2000

Abstract

Rapid, simple and accurate chromatographic (HPLC) and spectrophotometric methods for the determination of zopiclone in tablets were elaborated. Acetonitrile was found to be a suitable extraction solvent. The samples were chromatographed on Nova-Pak C18 column and UV detection at 304 nm. The elution was achieved isocratically with a mobile phase of 0.067 M phosphate buffer pH 7.95 — acetonitrile (55:45, v/v). Diazepam was applied as an internal standard. The method was validated for precision, linearity, accuracy and limit of detection. The recovery (mean ± SD) in HPLC was 99.85 ± 0.04% and in the UV-spectrophotometry 100.08 ± 0.09%. © 2000 Published by Elsevier Science B.V.

Keywords: Zopiclone; Reversed-phase chromatography; UV-spectrophotometry; Analysis in tablets

1. Introduction

Zopiclone,4-methyl-1-piperazinecarboxylic acid 6-(5-chloro-2-pyridinyl)-6,7-dihydro-7-oxo-5H-pyrrolo[3,4-b]-pyrazin-5-yl ester (Fig. 1a) is a non-benzodiazepine hypnotic drug possesing a short duration of action and few associated side effects. In adults, the therapeutic dose is 7.5 mg of zopiclone per os [1].

Zopiclone is widely used in therapy and therefore it is necessary to establish a simple and accurate method for its identification and quantitative determination in pharmaceuticals.

Techniques for the determination of zopiclone in tablets include polarographic [2,3] methods. Bouklouze et al. [2] described besides the polarographic method the quantitative analysis of zopiclone in tablets using ion-selective electrode. In literature it was stated about one rapid, simple and accurate chromaography (HPLC) method [4] which gives the determining of zopiclone in tablets using LiChrospher-60 RP Select B column and UV detection at 303 nm. The mobile phase consisted of a 0.018 M buffer pH 4.55 with an ion-par agent (3.4 g/l monosodium hexanesulfonate)-acetonitrile-tetrahydrofuran (81:18:1, v/v/v).

In the present study, new, simple and selective HPLC and UV-spectrophotometric methods were

* Corresponding author. Tel.: +48-81-7410221; fax: +48-81-5328903.

0731-7085/00/$ - see front matter © 2000 Published by Elsevier Science B.V.
PII: S0731-7085(00)00379-4

elaborated for the determination of zopiclone in commercial dosage forms.

2. Experimental

2.1. Reagents

Zopiclone was obtained from Sigma (St Louis, MO), diazepam (the internal standard) was purchased from Pharmaceutical Works 'Polfa' (Tarchomin, Poland). Imovane (7.5 mg of zopiclone) tablets (Theraplix Rhône–Poulenc Rorer, France) were used.

Acetonitrile LiChrosolv® for chromatography (E. Merck, Darmstadt, Germany) was applied. All the other reagents were of analytical grade. The water needed in the experiment was double distilled.

2.2. Instrumentation

2.2.1. Liquid chromatography

A Waters HPLC system (Milford, MA, USA) consisting of a Model 515 high-pressure pump, and a Model 2487 variable wavelength detector (UV-VIS) dual λ absorbance was used. Manual injections were made using a Rheodyne injectable valve (20 μl loop). The detector wavelength was set at $\lambda = 304$ nm. The chromatographic separations were performed at ambient temperature on a Nova-Pak C18 column (150 × 3.9 mm; d $p = 4$ μm) (Waters, Milford). The mobile phase was a mixture of acetonitrile and 0.067 M phosphate buffer pH 7.95 (45:55, v/v), filtered and degassed prior to use, and flowing at the rate of 1 ml/min. The data were collected and analyzed with Millenium 32 system software on Pentium MMX 166 MHz computer.

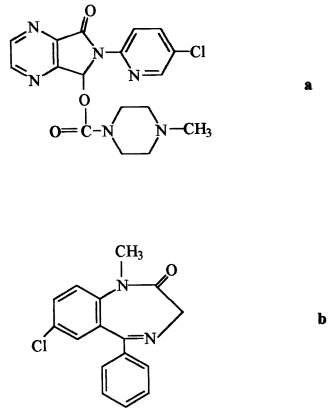

Fig. 1. Structures of (a) zopiclone and (b) diazepam (I.S.)

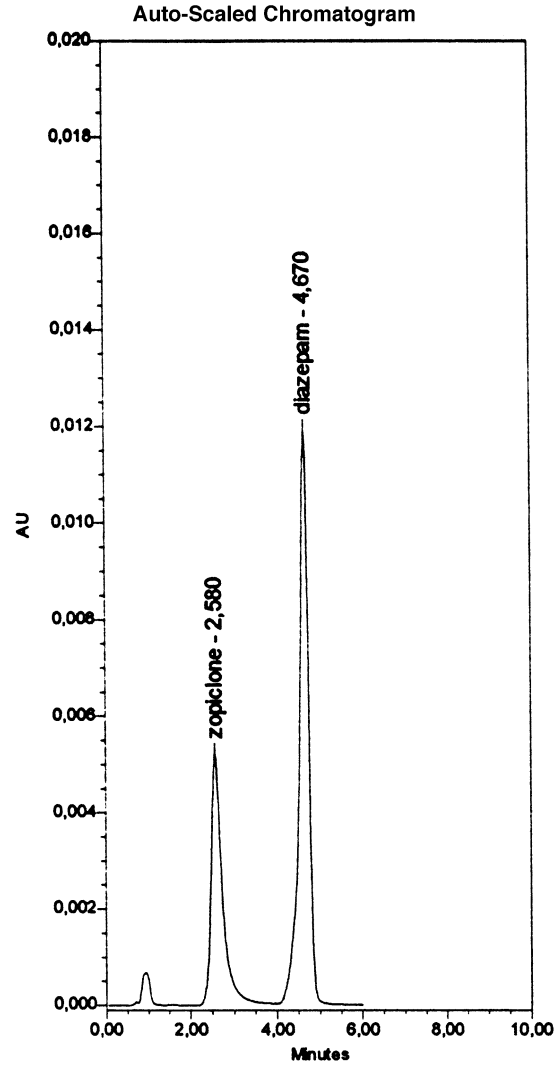

Fig. 2. Typical chromatogram of zopiclone and diazepam — internal standard under described HPLC conditions

2.2.2. Spectrophotometry

Analyses were performed on a Lambda 15 UV-VIS double-beam spectrophotometer (Perkin-

Table 1
Precision of the methods

No.	Amount found (mg in one tablet)	
	HPLC	Spectrophotometric method
1	7.436	7.509
2	7.509	7.588
3	7.495	7.515
4	7.539	7.520
5	7.484	7.548
6	7.490	7.506
7	7.505	7.497
8	7.497	7.501
9	7.487	7.490
10	7.492	7.513
Mean	7.493	7.519
SD	0.026	0.029
C.V. (%)	0.35	0.39

Elmer Co GmbH, Germany) using 1-cm quartz cells. Hand-press LQ-850 (Epson, Germany) was used.

2.3. Solutions

Stock solutions (1.0 mg/ml) of zopiclone and diazepam (I.S.) were prepared by dissolving appropriate amounts of there substances in acetonitrile. These solutions were stable for at least 2 months at 4°C. The working solutions of 0.01 mg/ml for zopiclone and 0.10 mg/ml for diazepam were prepared by diluting the stock solutions with the acetonitrile.

2.4. Chromatographic method

2.4.1. Calibration procedure

From the working solution of zopiclone, volumes of 1.0–6.0 ml were pipetted.

2.0 ml of the working solution of diazepam (I.S.) was added to each sample and made up with acetonitrile to 10.0 ml. A volume of 20 μl of each sample was injected into the column. All measurements were repeated five times for each concentration. The calibration curve was constructed by plotting the peak area ratios of analyte to I.S. versus the respective drug concentration.

2.4.2. Extraction from tablets and quantification

Twenty tablets were accurately weighed and the average tablet mass was calculated.

The tablets were triturated to a fine powder and amounts equivalent to 1.0–6.0 mg (after a declaration) of zopiclone were extracted with acetonitrile in 100 ml volumetric flasks. Filtered 1.0 ml volumes of the extracts were transfered into 10 ml measuring flasks, 2.0 ml of I.S. solution (0.1 mg/ml) was added and made up to the mark with acetonitrile. Then, 20 μl volume of each sample was injected into the column. All measurements were repeated five times for each concentration.

2.5. Precision

The precision the elaborated methods has been estimated by the means of ten determination of zopiclone in powdered tablets.

Table 2
Recovery values obtained for the determination of zopiclone in model mixtures[a]

Model mixture (%)	HPLC		Spectrophotometric method	
	Found	C.V. (%)	Found	C.V. (%)
I (50)	99.93	0.14	99.80	0.31
II (100)	99.85	0.11	100.11	0.15
III (150)	99.77	0.19	100.33	0.26

[a] Results are the average of six determinations and are expressed as a percentage of the zopiclone added.

2.6. Accuracy

The accuracy of the methods was shown by analyzing the model mixtures which were obtained by adding known amounts of zopiclone to placebo.The model mixtures contained 50 (I), 100 (II) and 150% (III) of zopiclone in comparing to the labelled tablet amount. For each model mixture it has been made six determination of zopiclone.

2.7. Spectrophotometric method

The absorptivity of zopiclone in acetonitrile was examined in the range 200–400 nm and the λ_{max} value (position of maximum absorbance of a peak) were recorded.

The spectrum exhibits a maximum at 304 nm and the calculated absorbance coefficient at this wavelength was $a_{1\,cm}^{1\%} = 420$.

To each of five 100 ml volumetric flasks about 15 mg of the powdered tablets were weighed, 50 ml of acetonitrile was added, shaked mechanically and completed to the mark, mixed and filtered. The absorbance was measured at 304 nm and the content of zopiclone was calculated using an absorbance coefficient.

3. Results and discussion

A reversed-phase isocratic procedure was proposed as a suitable method for the analysis of zopiclone in tablets. A mixture of 0.067 M phosphate buffer pH 7.95 — acetonitrile (55:45, v/v) at a flow rate of 1 ml/min was found to be an appropriate mobile phase allowing adequate and rapid separation of analyte and the internal standard (retention times 2.60 and 4.67 min, respectively). Diazepam, chosen as an internal standard, was clearly separated from zopiclone, and the total analysis time was 6.0 min. As shown in Fig. 2 the substances were eluted forming well shaped, symmetrical single peaks, well separated from the solvent front.

For quantitative applications, a linear calibration curve was obtained over the working concentration range 1–6 μg/ml. The parameters of the calibration graph were (mean ± SD)$y = 0.01277$ (± 0.00004)$x - 0.00386$ (± 0.00011); where y, peak area ratio of zopiclone to that of the I.S. and x, concentration of zopiclone in ng per 20 μl; correlation coefficient r, 0.9997 (± 0.0003) C.V., 0.03%. The results indicate a good linear proportionality between the detector response and the concentration of zopiclone.

Acetonitrile was chosen for the extraction from tablets because it is an excellent solvent for both the analyte and the internal standard and is suitable for the reversed phase made of chromatography.

The selectivity of the chosen chromatographic system was also ascertained. Excipients, i.e. dibasic calcium phosphate, hydroxypropylcellulose, lactose, magnesium stearate, polyethylene glycol, wheat starch showed no interferences with the determination of zopiclone and the internal standard.

The precision of the elaborated methods is given in Table 1. In order to verify the accuracy of the described methods, recovery studies were carried out by analyzing model mixtures of zopiclone. The recovery of zopiclone was evaluated from 50 to 150% of the labelled tablet amount. The accuracy of the methods is given in Table 2.

The limit of detection was 2 ng/ml (coefficient of variation C.V. = 6.7%).

The described HPLC method of determination of zopiclone in tablets is precise, sensitive and accurate. The advantages of the proposed method are its short analysis time and a simple procedure for sample preparation.

Results of the determinations of zopiclone show good precision and accuracy of the spectrophotometric method.

When Student's t test at the 95% confidence level was applied to compare the results obtained by the HPLC and the spectrophotometric method, the calculated t values did not exceed the tabulated one.

The rapid, simple and fairly reliable proposed methods were employed for the determination of zopiclone in tablets.

The satisfying recoveries and low coefficients of variation confirm the suitability of both proposed methods for the routine analysis of zopiclone in tablets.

References

[1] Y. Gaillard, I.P. Gay-Montchamp, M. Ollagnier, J. Chromatogr. 619 (1993) 310–314.
[2] A.A. Bouklouze, I.C. Vire, G.C. Quarin, I.M. Kauffmann, Electroanalysis 6 (1994) 1045–1050.
[3] I.A. Squella, I.C. Sturm, L.A. Alvarez, L.I. Nunez, J. AOAC Int. 77 (1994) 768.
[4] I.P. Bounine, B. Tardif, P. Beltran, D.J. Mazzo, J. Chromatogr. A 677 (1994) 87–93.