Journal of Chromatography, 380 (1986) 211–215
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3137

Note

Determination of zopiclone in plasma by liquid chromatography with application to steady-state monitoring

LAWRENCE G. MILLER, BARBARA W. LEDUC and DAVID J. GREENBLATT*

Division of Clinical Pharmacology, Departments of Psychiatry and Medicine, Tufts University School of Medicine and New England Medical Center, 171 Harrison Avenue, Boston, MA 02111 (U.S.A.)

(First received December 20th, 1985; revised manuscript received February 20th, 1986)

Zopiclone is a non-benzodiazepine hypnotic with a reported rapid onset of action and few associated side-effects [1, 2]. Several methods have been described to detect zopiclone in plasma, all involving high-performance liquid chromatography (HPLC) with spectrofluorometric detection. Prior methods have limited sensitivity [3] or are time-consuming owing to extraction procedures and long chromatographic retention times [4, 5]. We have designed a simple and sensitive method using reversed-phase HPLC and spectrofluorometry which gives a lower limit of detection of 2 ng/ml and is applicable to measurement of plasma zopiclone concentrations in humans following therapeutic doses.

EXPERIMENTAL

Materials

Pure samples of zopiclone, the internal standard (29481 R.P.), and two possible metabolites, the N-1-oxide (29753 R.P.) and the N-1-desmethyl analogue (32273 R.P.), were provided by Ives Labs. (New York, NY, U.S.A.; Fig. 1). All other reagents, analytical-reagent grade or better, were purchased from commercial sources and used without further purification. Mobile phase components were filtered prior to and after mixing.

Apparatus and chromatographic conditions

The HPLC system consisted of a Waters Solvent delivery system and a

0378-4347/86/$03.50

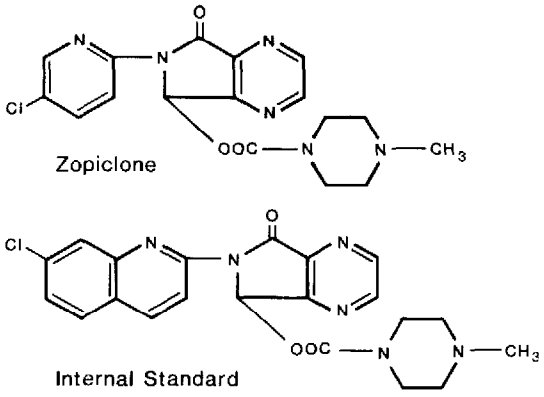

Fig. 1. Structures of zopiclone and the internal standard.

Perkin-Elmer 650-10S spectrofluorometer operated at excitation 305 nm and emission 420 nm (slit width: 10 and 13 nm, respectively). The separation system was a Radial-Pak C_{18} column housed in a radial compression module [6, 7] (Waters Assoc.). Samples were injected automatically with a WISP-710 injector (Waters Assoc.). The mobile phase was 0.02 *M* disodium hydrogen phosphate buffer, adjusted to pH 6.4 with phosphoric acid, with 0.8 ml/l di-*n*-butylamine—acetonitrile (50:50). The flow-rate was 1.2 ml/min. All analyses were performed at room temperature.

Stock solutions

Standard solutions were prepared by dissolving 100 mg of zopiclone, internal standard, or metabolites in 100 ml acetonitrile. The internal standard was unstable in methanol or water. Sequential dilutions to 1 μg/ml were then made. These solutions were stored in dark glass bottles at 4°C and were stable for at least two months.

Preparation of samples

A constant amount of the internal standard (75 ng) was added to each of a series of 15-ml glass round-bottom tubes with PTFE-lined screw-top caps. A 1.0-ml sample of unknown plasma was added to each tube. Calibration standards for zopiclone were prepared by adding 5, 10, 25, 50, 75, 100 ng of drug to consecutive tubes. Drug-free control plasma was added to each of the calibration tubes. One blank sample, taken from the subject prior to drug administration, was analyzed with calibration standards and each set of unknown samples.

For evaluation of possible interference by metabolites, varying concentrations were added to tubes containing zopiclone and the internal standard. Control plasma was then added as above.

Extraction procedure

A 50-μl aliquot of 0.01 *M* hydrochloric acid was added to each tube followed by 2 ml of dichloromethane—isoamyl alcohol (98:2). The tubes were agitated gently in the upright position on a vortex mixer for 5 s, then centrifuged at 20°C for 10 min at 400 *g*. The organic layer was transferred to

a 15-ml glass conical centrifuge tube and evaporated to dryness at 40° C at moderately reduced pressure. The samples were reconstituted into 0.15 ml acetonitrile and transferred to automatic sampling vials. Aliquots of 30—80 µl were then injected into the sample loop by the automatic sampler.

Clinical study

Subjects with insomnia were initially treated for four days with placebo. On day 5, either 7.5 or 15 mg of zopiclone were administered in the evening and blood samples were obtained immediately prior to the dose. Samples were subsequently obtained 9 h post-dosage and 24 h post-dosage (immediately before the next dose). Drug administration was continued in this manner for two weeks and additional samples were obtained on day 8 (9 h post-dosage), day 18 (24 h post-dosage), day 19 (9 h post-dosage) and day 20 (24 h post-dosage). Plasma was stored at −20° C until analysis was performed.

RESULTS

Evaluation of the method

Under the described chromatographic conditions, zopiclone and the internal standard gave symmetric well resolved chromatographic peaks (Fig. 2) with retention times 3 min for zopiclone and 4.8 min for the internal standard. The blank plasma after extraction consistently contains no interfering peaks. The relation between zopiclone concentrations and peak height ratio (versus

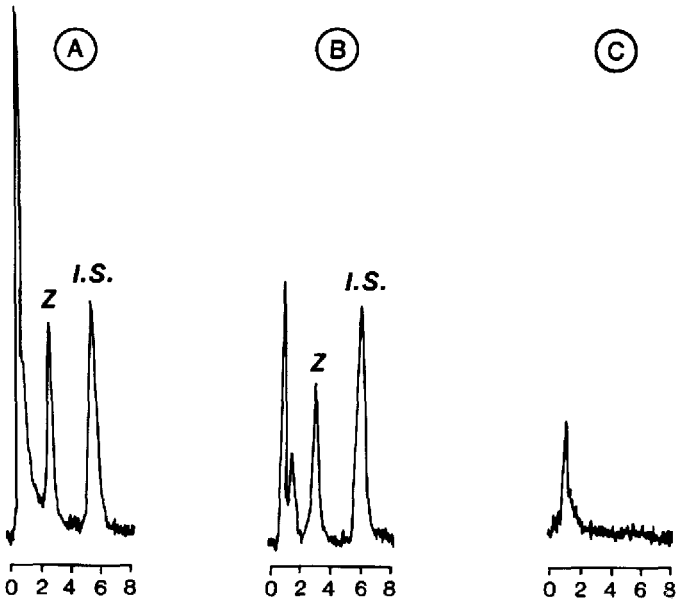

Fig. 2. (A) Chromatogram of an extract of a calibration standard containing 50 ng/ml zopiclone (Z) and 75 ng/ml internal standard (I.S.). (B) Chromatogram of a plasma sample from a subject taken 9 h after zopiclone (15 mg) administration (internal standard added). (C) Chromatogram of a blank plasma sample taken from a subject prior to zopiclone administration. The abscissa is retention time in min.

internal standard) is linear at least from 2 to 100 ng/ml ($y = 70.2x$). Correlation coefficients are always greater than 0.98. Relative standard deviation (S.D./$\overline{X}$, expressed in %) calculated by doing six replicate analyses of known standards (2, 5, 10, 25, 50, 75 ng/ml) were: 4.8% at 2 ng/ml, 5.2% at 5 ng/ml, 5.2% at 10 ng/ml, 3.4% at 25 ng/ml, 4.2% at 50 ng/ml, and 5.5% at 75 ng/ml. Minimum detectable concentration was 2 ng/ml (signal-to-noise ratio of 2:1). The between-day variability was assessed by inclusion of previously mixed quality-control samples of 75 ng/ml zopiclone during extractions and analyses. The relative standard deviation for six analyses was 3.3% (73.2 ± 2.4 ng). Residue analysis showed no appreciable drug after the initial extraction.

The N-1-oxide metabolite eluted at approximately 1.9 min and did not interfere with the zopiclone peak. The N-1-desmethyl analogue eluted immediately prior to zopiclone at 2.6 min. No interference with the drug peak was noted using up to 50 ng of the analogue.

Clinical study

Results of oral zopiclone administration to fifteen subjects at the specified times are presented in Table I. In five subjects receiving placebo no drug was detectable throughout. In the ten subjects receiving zopiclone, no drug was detectable before dosage began. In five subjects receiving 7.5 mg per dose, levels ranged from 18.4 to 35.2 ng/ml at 9 h post-dosage and were undetectable in all but one subject at 24 h post-dosage. In five subjects receiving 15 mg per dose, levels ranged from 25.8 to 68.1 ng/ml at 9 h post-dosage and were undetectable to 15.9 ng/ml at 24 h post-dosage. The N-1-oxide or N-1-desmethyl metabolites were not detectable in any sample.

TABLE I

PLASMA ZOPICLONE CONCENTRATIONS IN TWO STUDY GROUPS

Subject No.	Plasma zopiclone concentration (ng/ml)					
	Day 6 9 h post	Day 7 24 h post	Day 8 9 h post	Day 18 24 h post	Day 19 9 h post	Day 20 24 h post
Dose 7.5 mg						
1	19.3	N.D.*	28.6	N.D.	35.2	N.D.
2	18.4	N.D.	22.0	N.D.	21.1	N.D.
3	33.4	N.D.	23.2	N.D.	29.5	N.D.
4	33.6	8.9	34.6	N.D.	33.3	N.D.
5	18.6	N.D.	12.7	N.D.	27.5	N.D.
Mean ± S.D.	24.7 ± 7.2		24.2 ± 7.3		29.3 ± 4.9	
Dose 15 mg						
1	54.5	8.1	44.6	6.6	30.8	5.3
2	63.0	N.D.	31.0	N.D.	25.8	N.D.
3	67.3	5.6	68.1	15.9	66.9	10.5
4	28.9	8.0	36.2	11.5	39.8	N.D.
5	57.7	8.1	54.5	4.3	54.0	5.7
Mean ± S.D.	54.3 ± 13.4	6.0 ± 3.1	46.9 ± 13.3	7.7 ± 5.5	43.5 ± 15.1	4.3 ± 4.0

*N.D. = Not detectable (0 used in analysis).

DISCUSSION

This report describes a reliable and selective method for the quantitation of zopiclone in plasma using HPLC. The method has improved sensitivity and greater simplicity of sample preparation in comparison with other previously published techniques [3—5, 8]. These methods reliably detect 10 ng/ml, in contrast to the present method which has a lower limit of detection of 2 ng/ml. This method produces blank plasma samples that are consistently free of contaminants in the areas corresponding to the retention times for zopiclone and the internal standard.

The extraction procedure is simple and rapid. An acidic extraction from plasma and concentration into a small volume for autoinjection into the HPLC system are employed. Care must be taken to avoid degradation of the internal standard [4]. Stock solutions should be prepared in acetonitrile rather than methanol or water. During the extraction, the internal standard should be allowed to remain in aqueous solution for as brief a period as possible before addition of the organic phase. In addition, a very brief (5 s) period of agitation should be used to avoid emulsification. Should this occur, the emulsion may be resolved by freeze-thawing, or by addition of small additional amounts (0.5—1.0 ml) of the extractant.

The method is selective in that the N-1-oxide metabolite elutes clearly prior to zopiclone. The N-1-desmethyl analogue was separable from zopiclone at the amounts used. If greater quantities of this analogue were present, some interference might occur. Evidence from this study as well as prior evidence indicates that the desmethyl analogue is not present in human plasma [3, 4].

The method was used to evaluate zopiclone levels in humans during repeated dosage in a therapeutic setting. No drug was found in five patients receiving placebo, confirming the selectivity of the method. At 9 h after dosage, mean plasma levels in the groups receiving 7.5 and 15 mg of zopiclone were approximately proportional to the doses. Levels at 24 h showed low concentrations or were undetectable indicating little accumulation. This method is well suited to further studies of pharmacokinetics and in clinical trials of zopiclone.

ACKNOWLEDGEMENTS

This work was supported in part by Grants MH-34223 and AG-00106 from the United States Public Health Service.

REFERENCES

1 D. Wheatley, Br. J. Psych., 146 (1985) 312.
2 A.N. Nicholson and B.M. Stone, Int. Pharmacopsych., 17 (Suppl. 2) (1982) 92.
3 C. Stanley, P. Mitchell and C.M. Kaye, Analyst, 110 (1985) 83.
4 J. Gaillot, D. Heusse, G.W. Hougton, J. Marc-Aurele and J.F. Dreyfus, Int. Pharmacopsych., 17 (Suppl. 2) (1982) 76.
5 G. Caillé, P. duSouich, J. Spenard, Y. Lacasse and M. Vezina, Biopharm. Drug Dispos., 5 (1984) 117.
6 D.J. Greenblatt, R.M. Arendt and A. Locniskar, Arzneim.-Forsch., 33 (1983) 1671.
7 D.J. Greenblatt, R. Matlis, D.R. Abernethy and H.R. Ochs, J. Chromatogr., 275 (1983) 450.
8 G. Parker and C.J. Roberts, Br. J. Clin. Pharmacol., 16 (1983) 259.