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Note

Identification and quantification in plasma of zopiclone by gas chromatography with nitrogen–phosphorus detection

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Zopiclone is a non-benzodiazepine hypnotic drug that has been shown in insomniac patients to possess rapid onset of action and few associated side-effects [1–3]. In adults, the therapeutic dose is 7.5 mg of zopiclone per os [4] and plasma levels usually lie in the range 20–80 ng/ml [5]. Several methods have been described to detect zopiclone in plasma, all involving high-performance liquid chromatography (HPLC) coupled with fluorescence detection [6–9].

To the best of our knowledge, no gas chromatographic (GC) method has been described for assaying zopiclone. Therefore, we have developed a new direct GC method, which has permitted the analysis of a great number of clinical specimens without column reconditioning.

EXPERIMENTAL

Chemicals and reagents

Zopiclone (Rhône-Poulenc Santé, Courbevoie, France) and the internal standard (I.S.) diazepam (Roche, Neuilly-sur-Seine France) were gifts from the manufacturers. Solvents and chemicals were HPLC and analytical grade, respectively. Stock solutions of zopiclone and I.S. (1 mg/ml) were prepared in

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methanol and stored at 4 °C. The zopiclone standard concentrations, obtained by dilution with methanol, were 5, 20, 50, 100, 200 and 300 ng/ml.

Phosphate buffer was a 1 *M* solution of potassium dihydrogenphosphate prepared with deionized water and adjusted to pH 5.5. Buffered solutions of pH 6.6 and 9.5 were prepared in the same manner and adjusted to the desired pH.

Chromatographic conditions

GC was performed on 1.80 m×2 mm I.D. glass column with 3% OV-17 on 100–120 mesh Chromosorb Q (Alltech, Milford, MA, U.S.A.). The GC system consisted of a Perkin-Elmer (8 500) chromatograph with a nitrogen–phosphorus detector and a Perkin-Elmer data collector (Sigma 15). The column, injector port and detector temperatures were 240, 290 and 300 °C, respectively. The nitrogen carrier gas pressure was 2.4 bar. Zopiclone was quantified by plotting peak-area ratios (drug/I.S.) against the concentration of standard to produce standard curves and comparing the results for the case samples with the curves.

Procedures

Plasma (2 ml), 1 ml of phosphate buffer (1 *M*, pH 5.5), 40 μl of diazepam (400 ng/40 μl) and 5 ml of dichloromethane were pipetted into a 20-ml Pyrex centrifuge tube. After vortex-mixing and centrifugation, the solvent phase was evaporated to dryness at 45 °C in Speed Vac concentrator (Savant Instruments, Farmingdale, NY, U.S.A.). The residue was dissolved in 20 μl of methanol, and 1 μl was injected into the column.

RESULTS AND DISCUSSION

The procedure as described has been found to be both sensitive and specific for the analysis of zopiclone. Retention times were 3.80 and 4.98 min for zopiclone and I.S., respectively. Drug-free human plasma produced clean GC traces with no interfering peaks. Fig. 1 is a typical chromatogram of an extracted plasma obtained from a subject under zopiclone treatment. Diazepam, chosen as an internal standard, was clearly separated from zopiclone, and the total analysis time was 7.5 min. There is no report in the literature of simultaneous clinical treatment with zopiclone and diazepam, therefore the use of diazepam, although it is a widely prescribed drug, seems not to be a drawback. Nevertheless, its furtuitous presence can be easily detected by an abnormal increase of the chromatogram peak area of the internal standard and the presence of desmethyldiazepam, its metabolite. In this case an alternative I.S., e.g. clobazam, would be required.

The calibration curve was linear over the range 5–300 ng/ml for zopiclone. The mathematical expression ($n=4$) and the correlation coefficient of zopi-

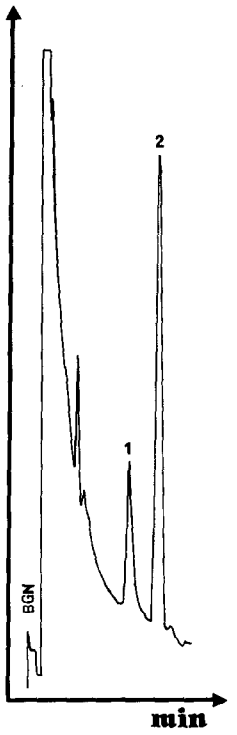

Fig. 1. Gas chromatogram of an extract of a plasma sample obtained from a subject under zopiclone treatment; zopiclone concentration = 48 ng/ml. Peaks: 1 = zopiclone; 2 = I.S.

TABLE I

ACCURACY OF THE DETERMINATION OF ZOPICLONE IN PLASMA

Concentration given (ng/ml)	Concentration found after extraction (mean ± S.D., $n=6$) (ng/ml)	Error (%)
5.0	5.6 ± 0.3	12.0
20.0	18.2 ± 0.8	−9.0
50.0	47.7 ± 10.1	−4.6
100.0	100.8 ± 7.3	0.8
200.0	200.9 ± 13.9	3.4
300.0	286.5 ± 3.1	−4.5

clone are $y = 0.98x + 0.04$ and $r = 0.998$, respectively. The results indicate a good linear proportionality between the detector response and the concentration of zopiclone in plasma.

The accuracy of the method was assessed by carrying six replicate samples

TABLE II

REPRODUCIBILITY IN PLASMA FOR ZOPICLONE

Concentration (ng/ml)	Within-run precision ($n=9$) (%)	Day-to-day precision ($n=6$) (%)
50	6.8	7.9
200	4.8	5.3

TABLE III

COMPARISON OF ORGANIC SOLVENTS FOR THE EXTRACTION OF ZOPICLONE FROM PLASMA

Solvent	Zopiclone recovery (%)		
	pH 5.5	pH 6.6	pH 9.5
Diethyl ether	65.0	30.2	27.0
Chloroform	31.7	77.0	40.4
Dichloromethane	81.6	43.2	34.0
Cyclohexane	26.5	45.6	64.3
Ethyl acetate	17.9	45.6	64.3
Diethyl ether–cyclohexane (2:1)	5.2	65.6	60.2
Hexane–dichloromethane (2:1)	22.2	37.6	16.5
Dichloromethane–isoamyl alcohol (98.5:1.5)	20.6	27.0	18.3

TABLE IV

RETENTION TIMES OF TESTED BENZODIAZEPINES

Drug	Retention time (min)	Drug	Retention time (min)
Estazolam	1.40	Temazepam	7.83
Metazepam	2.33	Prazepam	8.01
Oxazepam	3.44	Tetrazepam	8.03
Zopiclone	3.80	Alprazolam	8.26
Lorazepam	4.39	Bromazepam	8.30
Diazepam	4.98	Flunitrazepam	8.50
N-Desmethyldiazepam	6.98	Nitrazepam	12.04
Clobazam	7.36	Clonazepam	14.70

of six concentrations of zopiclone (5, 20, 50, 100, 200 and 300 ng/ml) through the entire procedure in one analysis day. The results are reported in Table I. Day-to-day precision was studied by adding zopiclone to blank plasma at therapeutic and toxic concentrations Analyses were performed every day over 3

weeks. The precision was found to be 4–8% (Table II). The within-run studies are also summarized in Table II.

During the initial stages of this work, various common extraction solvents were used to recover zopiclone from plasma. Extraction recoveries were determined by comparing the representative peak areas of extracted plasma (50 ng/ml zopiclone) with the peak areas of methanolic standards at the same concentration (external standard quantification). The results are presented in Table III. Dichloromethane was selected because it produced emulsion-free extracts with an acceptable extraction efficiency.

The limit of detection was determined by spiking plasma with decreasing concentrations of zopiclone until a response equivalent to three times the background noise was observed. The lower limit of detection was found to be 2 ng/ml. This detection limit is adequate for clinical analyses.

Some benzodiazepines were tested on the OV-17 column, since they can be associated with zopiclone in people who attempt suicide. All the compounds examined were eluted at retention times different from those of zopiclone and I.S., except oxazepam and nitrazepam (Table IV).

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