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Note

High-performance liquid chromatographic determination of zolpidem, a new sleep inducer, in biological fluids with fluorimetric detection

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Zolpidem, 2-(4-methylphenyl)-N,N,6-trimethylimidazo[1,2-*a*]pyridine-3-acetamide (Fig. 1), is a new sleep inducer, with a fast onset of action and a short half-life [1—3]. It is a weak base (pK_a 6.2), its solubility in water as the tartrate salt is 23 mg ml^{-1} at 20°C, and its octanol—water partition coefficient (*P*), measured by high-performance liquid chromatography (HPLC) [4] and expressed as its logarithm, is log *P* = 2.43. As a powder, zolpidem hemitartrate is stable when exposed to heat and humidity. Its methanolic solution develops a yellow colour after several hours in the presence of light and oxygen.

As plasma concentrations were expected to be in the low nanogram range after 10- and 20-mg oral doses, a sensitive analytical method was developed to determine zolpidem in biological fluids. The extended aromatic structure of zolpidem permits the use of a fluorescence detection method.

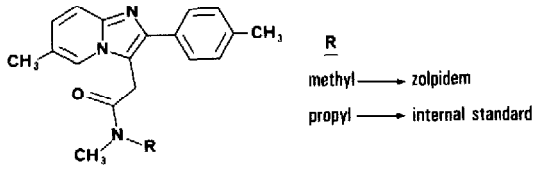

Fig. 1. Structures of zolpidem and of its internal standard (as bases).

EXPERIMENTAL

Standards and reagents

Zolpidem hemitartrate and its internal standard, N,6-dimethyl-2-(4-methyl-

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phenyl)-N-propylimidazo[1,2-*a*]pyridine-3-acetamide (Fig. 1), were synthesized by the Chemistry Department of LERS (Bagneux, France). Analytical reagent grade potassium dihydrogen phosphate, potassium hydroxide, and LiChrosolv acetonitrile, used for the mobile phase, were purchased from Merck (Darmstadt, F.R.G.). Analytical reagent grade, non-stabilized diethyl ether, used for extraction, was obtained from Carlo Erba (Milan, Italy).

Stock solutions

Stock solutions of zolpidem hemitartrate (or its internal standard as the methanesulphonate salt) in methanol (100 μg ml^{-1}) were prepared weekly.

Instruments

The chromatographic system consisted of an LDC Constametric II G pump, connected to a WISP 710B automatic injector (Waters Assoc.), a spectrofluorimetric detector (Kontron SFM 23B) and a Perkin-Elmer 56 recorder. Integration was performed by the 3357 Laboratory Automation System program implemented on a Hewlett-Packard HP 1000 computer.

Chromatographic conditions

Separations were performed at room temperature with a Spherisorb ODS-2 5-μm column (15 cm × 4.6 mm I.D.) (Phase Separations, Queensferry, U.K.) purchased from SFCC (Gagny, France). The flow-rate of the mobile phase, a 70:30 mixture of acetonitrile and 0.05 *M* potassium dihydrogen phosphate (pH 6), was 1 ml min^{-1}. The excitation and emission wavelengths were 254 and 390 nm, respectively.

Optimization of the extraction pH and solvent

In order to determine the optimum extraction pH, plasma samples (1 ml) spiked with 3 ng of zolpidem hemitartrate were extracted with diethyl ether (7 ml) at pH values from 2 to 13 after addition of 1 ml of the appropriate buffer. A 5-ml volume of the ether phase was evaporated to dryness at 37°C under a gentle stream of nitrogen. The residue was dissolved in 800 μl of an injection solution [acetonitrile—0.05 *M* potassium dihydrogen phosphate (pH 6) (40:60)], 500 μl of which were injected and analysed as described above.

For the evaluation of the best extraction solvent, plasma samples (1 ml) spiked with 3 ng of zolpidem hemitartrate and buffered at pH 12 were extracted by various organic solvents according to the method described below.

Extraction procedure

In a conical tube containing 50 ng of internal standard, the plasma or blood sample (500 μl) was adjusted to pH 11.5—12.5 by addition of 250 μl of 0.25 *M* potassium hydroxide solution. Zolpidem was extracted by shaking with non-stabilized diethyl ether (7 ml) for 30 min. Following centrifugation (1000 *g* for 5 min at —20°C), the upper organic phase (6.5 ml) was transferred to a second tube and evaporated to dryness at 37°C under a gentle stream of nitrogen. The residue was then dissolved in 1 ml of methanol, and 20 μl were injected on to the column via an automatic injector.

RESULTS

Extraction

As expected from the pK_a value, the maximum extraction yields were obtained at pH values higher than 6 (Fig. 2). From pH 6 to 13 the recovery of zolpidem was ca. 94%. Extraction of 500 μl of plasma as described in the Experimental section showed that a cleaner chromatogram was observed between pH 11 and 13. Therefore, the pH of the plasma or blood was adjusted to 11.5—12.5 prior to extraction.

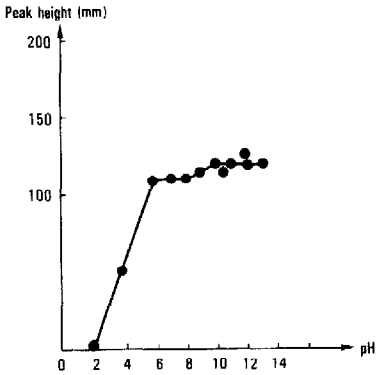

Fig. 2. Effect of pH on the extraction of zolpidem. The yield is expressed as the chromatographic peak height.

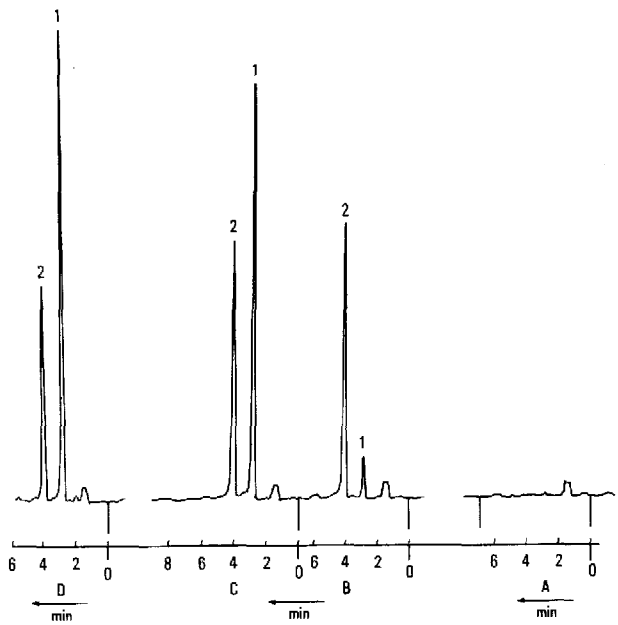

Fig. 3. Chromatograms of (A) blank plasma, (B) plasma spiked with zolpidem (8 ng ml^{-1}) and its internal standard, (C) plasma spiked with zolpidem (90 ng ml^{-1}) and its internal standard, (D) a plasma sample from a healthy volunteer following administration of a 20-mg oral dose of zolpidem hemitartrate. Peaks: 1 = zolpidem; 2 = internal standard. For chromatographic conditions, see text.

The following solvents were ranked by increasing extraction yield as follows: hexane < toluene < chloroform < methylene chloride < diethyl ether. Diethyl ether was chosen as it also gave minimal interferences from endogenous compounds.

Quantitation

Chromatograms obtained from a blank plasma, a plasma spiked with zolpidem hemitartrate and its internal standard and from a patient sample (following administration of zolpidem) are shown in Fig. 3. Similar chromatograms were obtained with blood or urine samples. The peaks corresponding to zolpidem and its internal standard were well resolved without any interference from endogenous compounds. The retention times were 2.8 and 4.0 min for zolpidem and its internal standard, respectively (the heights equivalent to a theoretical plate were 54 and 50 μm, respectively).

Calibration curves obtained after extraction from blood (or plasma) were linear between 1 and 400 ng ml^{-1}. The practical sensitivity of the method was 1 ng ml^{-1} for blood or plasma samples, but 0.5 ng ml^{-1} could be detected with a signal-to-noise ratio of 3.

Quality controls

Reproducibility of the method was evaluated by means of an internal quality

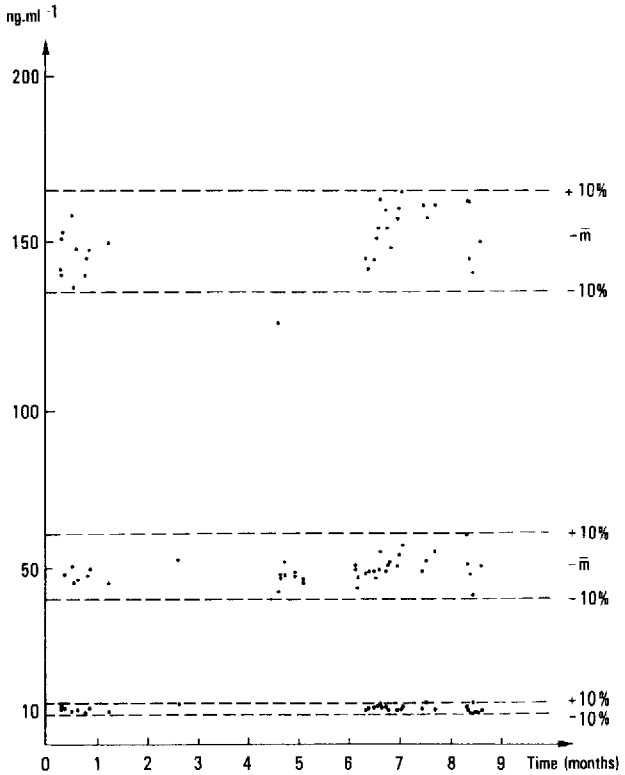

Fig. 4. Results of the internal quality control over an eight-month period at 10, 50 and 150 ng ml^{-1} ($\bar{m}$ = mean). At 50 ng ml^{-1}, measurements were obtained over a thirteen-month period, but only the results for the first eight months are shown.

control. Pig blood samples, spiked with zolpidem at 10 and 150 ng ml^{-1}, were analysed over an eight-month period and at 50 ng ml^{-1} over thirteen months. Individual results are shown in Fig. 4. For the 10 and 150 ng ml^{-1} concentrations the experimental mean values (± S.D.) were 10.4 ± 0.75 ng ml^{-1} (n = 32) and 150.7 ± 9.5 ng ml^{-1} (n = 33) with a coefficient of variation (C.V.) of 7.2 and 6.3%, respectively. For the 50 ng ml^{-1} samples the experimental mean was 53.0 ± 5.0 ng ml^{-1} with a C.V. of 9.5%.

As shown in Fig. 4, only one data point fell outside the 10% range during the eight-month period.

Stability of zolpidem

The stability of zolpidem in plasma or blood samples at 10 ng ml^{-1} was measured at 37°C for up to 24 h. No significant degradation was found during this time.

Spiked blood samples, adjusted to pH 12, were left at room temperature for 0.5, 1 and 4 h, then extracted as usual. No noticeable variation in peak heights was observed, which means that the drug is stable under the extraction conditions for at least 4 h.

Applications

As samples can be injected every 6 min, a large number of samples may be analysed with an automatic injector. However, the limiting factor is the number of samples that can be extracted. In our hands, sixty samples could be processed in a routine working day, and the method has been used to analyse several thousands of samples over the past two years.

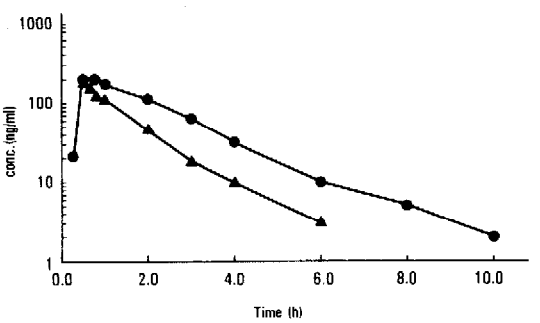

Fig. 5. Plasma concentrations of zolpidem measured following administration of a single 20-mg oral (●) and a single 8-mg intravenous (▲) dose of zolpidem hemitartrate.

As an example, blood concentrations observed in one healthy volunteer following oral and intravenous administration of zolpidem (20 and 8 mg, respectively) are reported in Fig. 5. Blood levels peaked at ca. 200 ng ml^{-1} 30 min after oral administration and decayed with a half-life of ca. 2 h.

DISCUSSION

The intrinsic fluorescence of zolpidem is such that a limit of sensitivity of 1 ng ml^{-1} can be attained without difficulty. Starting with 500 μl of plasma

only 2% of the extract is actually injected; that is to say that the determination is performed with 10 μl of plasma. So it seems likely that direct plasma injection should be feasible. Preliminary experiments have shown that the use of a pre-column is required. We are currently developing a fully automated method using plasma samples.

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