Journal of Chromatography, 377 (1986) 368—372
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3005

Note

Determination of lormetazepam in plasma by gas chromatography and electron-capture detection

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(First received May 10th, 1985; revised manuscript received December 3rd, 1985)

Lormetazepam [7-chloro-5-(2-chlorophenyl)-3-hydroxy-1-methyl-1,3-dihydro-1H-1,4-benzodiazepin-2-one] (Fig. 1) is a benzodiazepine recently introduced as a hypnotic agent, marketed as Minias® [1—3]. Pharmacokinetic and metabolism studies in humans were first performed by administration of the ^{14}C-labelled drug [4].

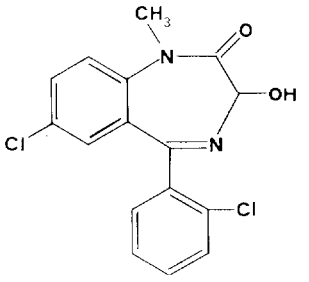

Fig. 1. Structural formula of lormetazepam.

0378-4347/86/$03.50

In further studies, the analysis of lormetazepam and its major metabolite in plasma has been carried out with radioimmunoassay [5, 6] and radioreceptorassay [7]. Radioimmunologic determination needs the development of an antiserum; furthermore, cross-reactions with other benzodiazepines and other compounds can occur [5] if the sample is not first separated by liquid chromatography [6]. A radioreceptor method, which has been employed to measure the lormetazepam binding equivalent in plasma, accounts unspecifically for several benzodiazepines [7].

At present, no chromatographic methods are known for analysis of lormetazepam in blood. Measured plasma concentrations of drug after oral dosing of 2 mg in humans were below 25 ng/ml [4—7], therefore sensitive methods are necessary.

Our purpose was to find a sensitive, simple and specific method for the determination of lormetazepam in plasma samples. This paper describes a gas chromatographic procedure using electron-capture detection which requires a relatively simple extraction and derivatization procedure. The detection limit was 0.5 ng/ml and the recovery was ca. 95% with good reproducibility.

EXPERIMENTAL

Reagents and solutions

Lormetazepam and temazepam (internal standard) were purchased from Farmades (Rome, Italy). N,O-Bis(trimethylsilyl)acetamide (BSA) was obtained from Merck (Darmstadt, F.R.G.). All other chemical reagents and solvents were of analytical grade from Carlo Erba (Milan, Italy).

Stock solutions of benzodiazepines (1 mg/ml) in acetone were prepared weekly and stored at 5°C, protected from the light.

Borate buffer solution (pH 9.2) was prepared by dissolving 19.5 g boric acid—23.5 g potassium chloride—19.6 g sodium carbonate in 500 ml of water.

Gas chromatography

A Perkin-Elmer (Norwalk, CT, U.S.A.) Sigma 4 gas chromatograph equipped with a ^{63}Ni electron-capture detector and a 2.20 m × 4.0 mm I.D. glass column packed with 3% OV-17 on Chromosorb Q (100—200 mesh) was employed. The carrier gas was argon—methane (95:5) with a flow-rate of 45 ml/min. The oven temperature was 250°C and the temperature of the injection port and detector was 300°C.

Procedure

For extraction, 1 ml of plasma was pipetted into 12-ml tapered tubes containing 20 ng of temazepam (internal standard). Borate buffer (1 ml) and 5 ml of the mixture toluene—isoamyl alcohol (100:1.5, v/v) were added. The tubes were shaken for 10 min on a reciprocal shaker, then centrifuged for 5 min at 1500 *g*. A 4-ml aliquot of the organic layer was transferred into another tube and evaporated to dryness under a nitrogen flow at 55°C. The residues were reacted with 100 μl of BSA for 15 min at 50°C in the tapered tubes. Finally, the reagent was evaporated at 50°C under a nitrogen flow, and the residues were reconstituted in 100 μl of acetone, previously dried by the addition of

sodium sulphate. Aliquots (1—5 μl) of this solution were injected into the chromatograph.

Calibration, recovery and reproducibility

Calibration curves were constructed with blank plasma containing added concentrations (2, 5, 10, 20 and 30 ng/ml) of lormetazepam. As internal standard, 15 ng/ml temazepam was added to all samples, which were then extracted, derivatized and analysed as described. Calibration curves were drawn for lormetazepam concentration against the peak-height ratio of lormetazepam to temazepam.

Extraction recovery was determined in the following way. A plasma sample spiked with lormetazepam was extracted and the organic layer added to a known amount of temazepam. The same quantity of lormetazepam and temazepam as previously used were introduced into a tube (non-extracted). These samples were derivatized as described and injected into the gas chromatograph. The percentage recovery was calculated by comparing peak-height ratios obtained with the above-mentioned procedures (extracted versus non-extracted).

Reproducibility was assessed by replicate analysis of plasma samples containing 5, 10 and 20 ng/ml lormetazepam.

Blood samples (clinical application)

One healthy male volunteer (27 years of age and 76 kg body weight) was given one tablet containing 2 mg of lormetazepam, and 250 ml of water.

Blood samples were taken at 0, 0.5, 1, 2, 4, 8, 12, 24 and 48 h after drug administration. Samples were immediately centrifuged, and the plasma was transferred and stored at —30°C until analysis.

RESULTS

Chromatograms of blank plasma, processed according to the described method, do not show evidence of interfering peaks. The lormetazepam and temazepam peaks are well separated (Fig. 2). Application to the analysis of a plasma sample taken as mentioned above is shown in Fig. 2.

A linear relationship ($y = 0.02 + 0.05\ x$) between peak-height ratios versus drug concentrations was demonstrated in the range 0—100 ng/ml. Higher concentrations are not of therapeutic interest.

Recovery was 95 ± 4% ($n = 6$), with a repeatability of 5.1%. This method had a detection limit of 0.5 ng/ml, which was sufficient for pharmacokinetic studies.

Fig. 3 shows the time-course curve of lormetazepam concentration in the plasma of a subject administered with this drug. Experimental data were fitted by non-linear regression analysis according to $C = A\ (e^{-\alpha t} - e^{-\beta t})$. The exponential term coefficient (A) was 14.75 and the absorption rate constant (α) was 0.604.

Peak concentration (11.1 ng/ml) was found at 4.7 h after oral administration. Thereafter, levels decayed with an elimination rate constant (β) of 0.044 and an elimination half-life of 15.7 h. The area under the curve ($AUC^{0\rightarrow\infty}$) was 311.1 ng h/ml.

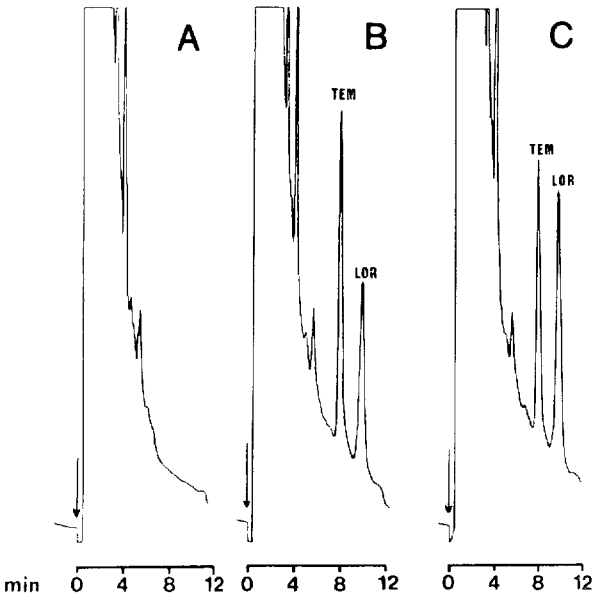

Fig. 2. Gas chromatograms of plasma extracts. (A) Blank plasma; (B) plasma sample obtained from a subject given 2 mg lormetazepam; (C) plasma spiked with lormetazepam (20 ng/ml) and temazepam (15 ng/ml). Peaks: TEM = temazepam; LOR = lormetazepam.

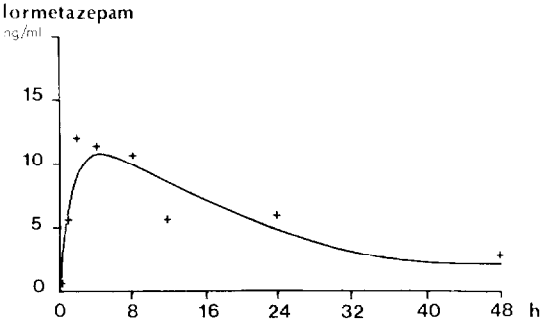

Fig. 3. Lormetazepam plasma concentration—time curve of a volunteer after administration of a 2-mg tablet. Pharmacokinetic data are given in the text.

DISCUSSION

In this method it was necessary to introduce a derivatization step, as the unchanged drug gives rise to tailed peaks and column absorption phenomena. The structurally related benzodiazepine, lorazepam, when chromatographed, undergoes a thermal rearrangement to quinazoline carboxaldehyde derivatives; however, no tailed peaks are observed [8].

We employed an extraction buffer almost identical to that introduced by De Silva et al. [9] and applied it to extract several benzodiazepines. The solvent mixture used here for extraction allowed us to obtain high recoveries without carrying interfering compounds. This could be attributed to the relatively low polarity of this mixture.

An adequate internal standard was necessary for this analysis, primarily because of the method of derivatization. Temazepam completely satisfies this need, as it is strictly analogous to lormetazepam and undergoes the same derivatization reaction with very similar chromatographic behaviour.

The main metabolic reaction is the formation of the non-reactive glucuronide derivative; less than 10% of the drug undergoes demethylation to lorazepam [4]. Considering this biotransformation, it can be said that the measurement of parent drug accounts for the only reactive compound in the blood.

The range of plasma concentration was found to be similar to that observed with lorazepam. Indeed, in healthy volunteers given 2 mg of lorazepam, the peak plasma concentration was 16.9 ng/ml as shown by Greenblatt et al. [10].

The use of an electron-capture chromatographic method for this analysis seems to be exclusive; in fact, the required sensitivity cannot be reached with a nitrogen-selective detector or other methods such as liquid or thin-layer chromatography.

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