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Note

Measurement of zipeprol in rat plasma by gas chromatography with nitrogen–phosphorus detection

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Zipeprol (Respilène) is a non-opiate, antitussive agent, but information about its kinetics is limited [1], mainly owing to the lack of suitable analytical methods for monitoring plasma levels. The method described here is based on gas chromatography (GC) using nitrogen–phosphorus selective detection (NPD). Because of its sensitivity and specificity, it offers general advantages over existing methods [1–4].

EXPERIMENTAL

Standards and solvents

Zipeprol [4-(2-methoxy-2-phenylethyl)-α-(methoxyphenylmethyl)-1-piperazineethanol] and pentazocine [1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocine-8-ol], which was chosen as an internal standard, were supplied by Eli Lilly (Indianapolis, IN, U.S.A.). The solvents diethyl ether, methanol, *n*-pentane and isopropanol (Merck, Darmstadt, F.R.G.) were used.

Stock solutions

Standard solutions in methanol of zipeprol (10 μg/ml) and pentazocine (10 μg/ml) were prepared by adding 0.1 mg of drug to 10 ml of methanol and were kept at 4°C. Under these conditions, the solutions were stable for several weeks. Working solutions of 1 and 0.1 μg/ml were prepared by sequential dilution.

Calibration graph and quantitation

A calibration graph for zipeprol was established by adding 1, 5, 25, 50, 100, 250 and 500 ng of zipeprol and 100 ng of pentazocine to 0.1 ml of drug-free plasma and processing the mixtures according to the method described below. The ratio of the peak area of zipeprol to that of pentazocine was used to calculate the calibration graph, the slope of which was used in the quantitation of zipeprol in plasma samples.

Extraction procedure

For each analysis 0.1 ml of plasma was used, to which 10 μl of 10 μg/ml pentazocine solution were added as an internal standard in a glass centrifuge tube. Then 7 ml of pentane–isopropanol (97:3) and 100 μl of 5 *M* sodium hydroxide solution were added and the tubes were stoppered and shaken mechanically for 20 min, then centrifuged for 5 min at 752 *g*. The organic layer was transferred into a 15-ml glass centrifuge tube, 0.5 ml of 0.2 *M* hydrochloric acid was added and extraction was performed by mixing for 10 min in a mechanical shaker. The solution was centrifuged for 5 min at 752 *g* and the organic layer was aspirated and discarded. The pH of the aqueous layer was adjusted to 14 with 150 μl of 5 *M* sodium hydroxide solution, re-extracted with 7 ml of distilled diethyl ether by shaking for 20 min and centrifuged for 5 min at 752 *g*. The organic layer was transferred into another tube and evaporated to dryness at reduced pressure and then dissolved in 50 μl of methanol, of which 3 μl were injected into the chromatograph.

Gas chromatography

All GC experiments were performed with a Hewlett-Packard (HP) 5890A gas chromatograph with a nitrogen–phosphorus detector, connected to an HP

TABLE I

GAS CHROMATOGRAPHIC CONDITIONS USED FOR THE DETERMINATION

Parameter	Condition
Column	HP fused-silica capillary, cross-linked 5% phenylmethyl silicone (SE-54); 16 m×0.2 mm I.D., film thickness 0.33 μm
Detector temperature	300°C
Initial temperature	100°C
Programming rate	20°C
Final temperature and time	300°C for 2 min
Carrier gas flow-rate	Helium at 1.5 ml/min
Auxiliary gas flow-rate	Helium at 20 ml/min
Hydrogen flow-rate	4.0 ml/min
Air flow-rate	100 ml/min
Splitting ratio	1·7

3392A integrator. All injections were made with an HP 7673A autosampler. GC conditions for the plasma sample analyses are given in Table I.

RESULTS

The gas chromatogram obtained from a plasma sample to which a known amount of zipeprol had been added is shown in Fig. 1 together with those ob-

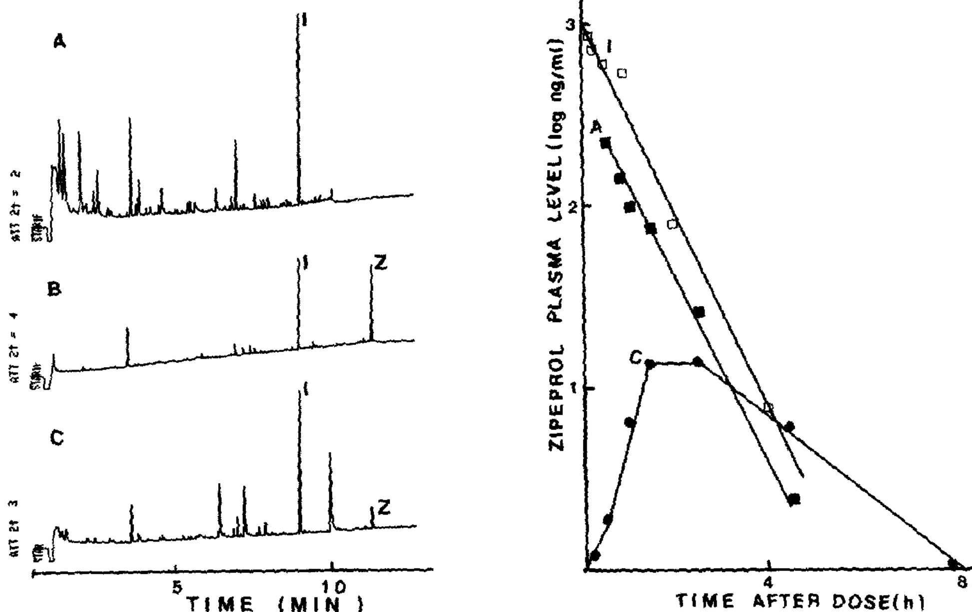

Fig. 1. Chromatograms of rat plasma extracts. (A) Blank plasma; (B) plasma to which 250 ng/ml zipeprol had been added; (C) plasma from a rat to which 20 mg/kg had been dosed orally. Peaks: I = internal standard (pentazocine); Z = zipeprol.

Fig. 2. Plasma profiles of zipeprol from rats after dosing by various methods: I, intravenously; A, oral dose as aqueous solution; C, oral dose as capsule.

TABLE II

PRECISION AND ACCURACY OF DETERMINATION OF ZIPEPROL IN 100 μl OF RAT PLASMA ($n=6$)

Concentration added (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)
50	53 ± 3	6.27
500	484 ± 16	3.33
2500	2500 ± 98	3.90

TABLE III

RECOVERIES OF ZIPEPROL ADDED TO 100 μl OF RAT PLASMA ($n=6$)

Concentration of standard (ng/ml)	Recovery (mean ± S.D.) (%)	Coefficient of variation (%)
50	71.3±9.3	12.9
500	79.7±2.9	3.7
2500	77.5±3.1	4.0

tained from blank plasma and plasma dosed orally. No interfering peaks from endogenous substances are present near the peaks of zipeprol and pentazocine.

The calibration graph for zipeprol from 5 to 500 ng/ml is linear. The line of best fit using the internal standard (pentazocine) is $y=0.025x+0.048$ ($r=0.9997$), where x is the analyte concentration and y is the peak-area ratio of zipeprol to pentazocine. The limit of detection was 5 ng/ml in 0.05–0.3 ml of plasma. The absolute sensitivity of the detector was 3 pg for zipeprol. The coefficient of variation of the method at several concentrations is reported in Table II and the recovery of zipeprol is reported in Table III.

This method is useful for bioavailability and pharmacokinetic studies in rats because the sensitivity is good and only a small sample size is required. The plasma concentration–time curve over 8 h is presented in Fig. 2. The absorption of zipeprol was very rapid and the calculated plasma half-life of zipeprol is 34 min.

In conclusion, a reliable and sensitive method for the determination of zipeprol in plasma using GC–NPD has been developed which appears to be both more sensitive and more convenient than previously reported GC methods [1,2]. It should be useful in pharmacokinetic studies or other applications in which low concentrations of zipeprol in biological samples must be determined. Our laboratory is currently utilizing this method to characterize the absorption of zipeprol according to different drug coating materials.

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