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Note

Determination of picogram levels of (*E*)-3-(9-chloro-6,11-dihydro-5H-pyrrolo(2,1-B)(3)benzazepin-11-ylidene)-N,N-dimethyl-1-propanamine (*Z*)-2-butenedioate (1:1) in plasma using capillary gas chromatography with nitrogen-selective detection

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(*E*)-3-(9-Chloro-6,11-dihydro-5H-pyrrolo(2,1-B)(3)benzazepin-11-ylidene)-N,N-dimethyl-1-propanamine (*Z*)-2-butenedioate (1:1) (herein referred to as I, Fig. 1) is a potential muscle relaxant candidate. In support of pharmacokinetic studies, it was desirable to have a reliable quantification of I at a level of 100 pg/ml of plasma to insure full mapping of plasma concentration–time courses. Therefore, a capillary gas chromatographic (GC) method with nitrogen-

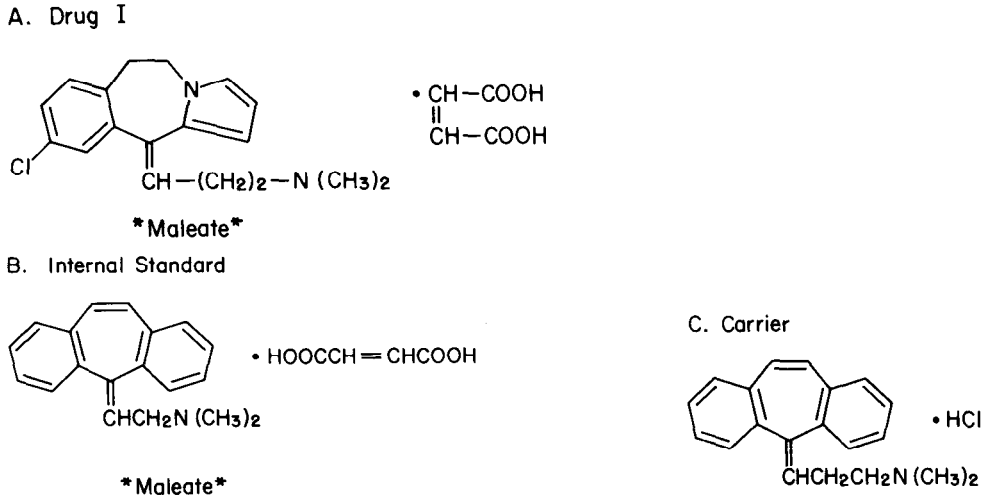

Fig. 1. Chemical structures of drug I (A), the internal standard (B) and the carrier (C).

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selective detection was developed which employs a double extraction of the biological specimen.

EXPERIMENTAL

Materials and reagents

Heptane (nanograde, Mallinckrodt, Paris, KY, U.S.A.), methanol (HPLC grade, Burdick & Jackson Labs., Muskegon, MI, U.S.A.), isoamyl alcohol (HPLC grade, Fisher Scientific, Fair Lawn, NJ, U.S.A.), drug-free human control plasma (Sera-Tec Biologicals, Harrisburg, PA, U.S.A.), sodium hydroxide (certified ACS, Fisher Scientific), LR101 concentration tubes (Laboratory Research, Los Angeles, CA, U.S.A.), and 13-ml polypropylene centrifuge tubes with caps (Sarstedt, Princeton, NJ, U.S.A.) were purchased from their respective suppliers. The drug (I), the internal standard [5-(2-dimethylaminoethylidene)-dibenzo[*a,e*]cycloheptatriene maleate salt] and carrier [3-(5H-dibenzo-[*a,d*]cyclohepten-5-ylidene)-N,N-dimethylpropanamine hydrochloride salt] (Fig. 1) were obtained from Merck (Rahway, NJ, U.S.A.).

Assay procedure

Plasma (1.0 ml), internal standard (10 ng), carrier (100 ng), 0.5 ml of 5 *M* sodium hydroxide and 6 ml heptane–isoamyl alcohol (90:10) were transferred to a 13-ml polypropylene centrifuge tube. The tube was stoppered and the contents shaken (15 min on a flat-bed shaker, 30 strokes per min) and then centrifuged (5 min at 3 *g*). The organic layer was transferred to an LR101 concentration tube and the heptane–isoamyl alcohol evaporated to dryness (50°C) under a stream of nitrogen. The remaining aqueous layer was extracted with a second volume of heptane–isoamyl alcohol, centrifuged and transferred to the same LR101 concentration tube as used during the first extraction and then evaporated to dryness. The residue was reconstituted in methanol (10 μl) and an aliquot (5 μl) injected onto the GC column.

Calculations

A standard curve of I in plasma was run daily with clinical specimens. The calibration curves for plasma were linear from 0.1 to 5 ng/ml. The equation for the resulting line was $y=0.0764x-0.00017$ (with $r^2=0.9991$). The peak-height ratio of the drug to internal standard from the unknown samples was employed to calculate their concentrations from the standard curve.

Instrumental

Analyses were performed on a Hewlett-Packard 5890 gas chromatograph equipped with a nitrogen–phosphorus detector. The capillary jet inside the detector was bored to 0.6 mm enabling placement of the capillary column end to within 1 mm of the collector bead, thereby maximizing sensitivity. A 50 m length, 0.20 μm film thickness, standard bore, CP Sil 20 CB liquid phase, Chrompack capillary column was used. A splitless glass liner was installed in the injector. The splitter was off and the septum purge was capped.

The data collection and integration were performed on a Hewlett-Packard 3357 laboratory automation system. The analog-to-digital converter was sampling at a rate of 4 Hz.

Instrumental conditions

The injection port and detector temperatures were set at 270°C and 300°C, respectively. The oven temperature program was: (a) 60°C initial temperature, 1 min hold; (b) 30°C/min to 180°C; (c) 5°C/min to 220°C, hold 7 min; (d) 1°C/min to 230°C. Flow-rates of hydrogen, air and helium (make-up) gases were 3.0, 100 and 20 ml/min, respectively. The helium (carrier) gas had a column head pressure of 1.03 bar.

RESULTS AND DISCUSSION

The within-day precision (repeatability) of the method is presented in Table I. A human plasma concentration–time profile is presented in Table II. Typical chromatograms are presented in Fig. 2.

With the thermionic bead operating current adjusted to give a baseline offset at 130 pA, a minimum of 10 pg of neat drug could be detected easily (signal-to-noise ratio > 10). In the presence of endogenous interferences, 250 pg of drug per ml of plasma could be quantified reliably as indicated by Table I. Since one half of the prepared plasma sample was injected, approximately 25 pg of the drug were

TABLE I

REPEATABILITY (WITHIN-DAY PRECISION, $n=6$)

Plasma concentration (ng/ml)	Coefficient of variation (%)
0.1	17.5
0.25	7.7
0.5	6.0
0.75	5.1
1.0	5.7
2.0	5.0
5.0	4.8

TABLE II

HUMAN PLASMA CONCENTRATION–TIME PROFILE FOLLOWING AN 8-mg ORAL DOSE OF I

Time (h)	Plasma concentration (ng/ml)
0	0
1	0.46
2	1.10
4	1.31
8	0.95

being quantified in the presence of endogenous interferences with absolute recovery of 50%.

A clean-up procedure which was more efficient in reducing the endogenous interferences relative to the drug would, in principle, yield an even more sensitive method. However, as with the structurally similar tricyclic antidepressants, the clean-up procedures can lead to losses of the drug due to adsorptive phenomena [1]. To minimize these drug losses, an extraction with heptane–isoamyl alcohol was employed for drug recovery with no back-extractions. Heptane was tried as a solvent for extraction, but yielded low drug recoveries. Isoamyl alcohol was

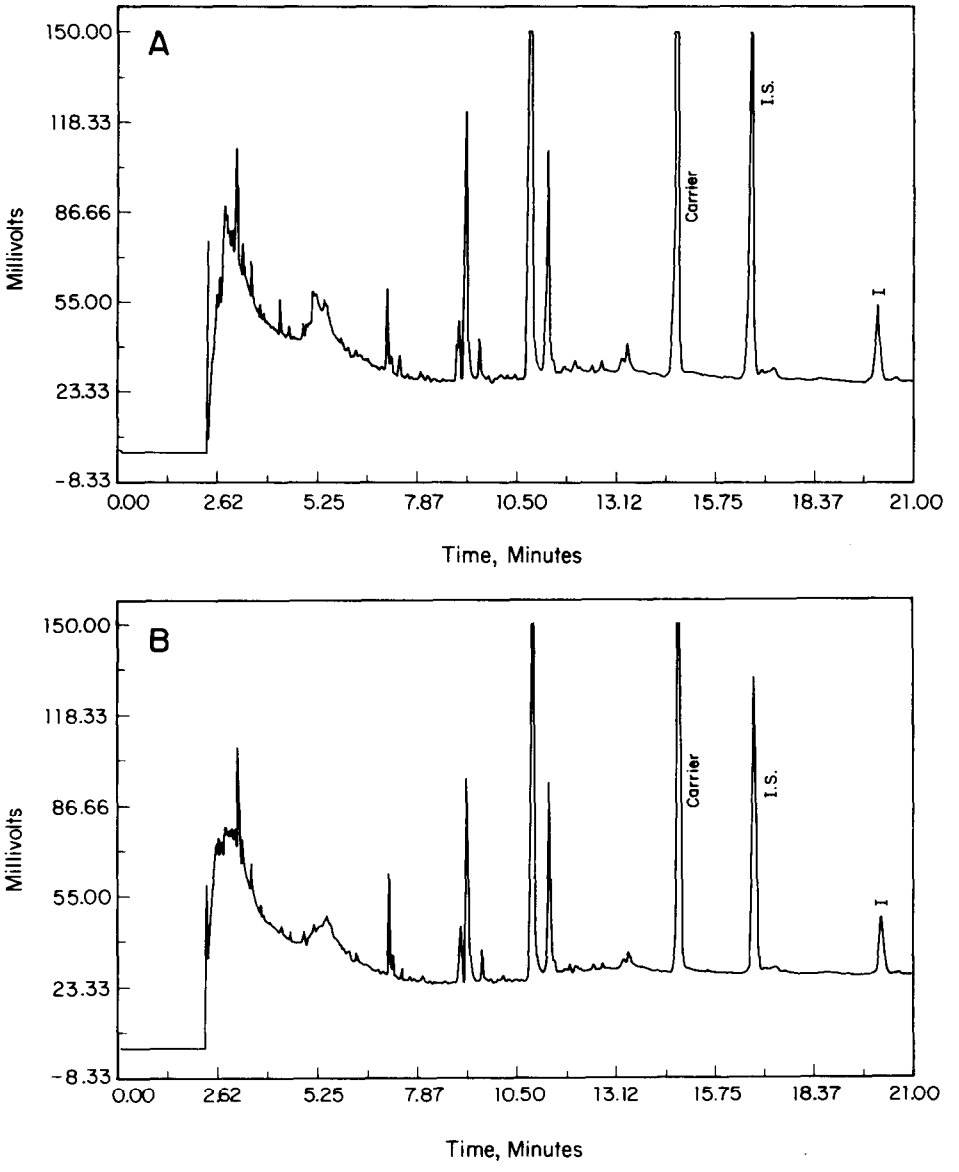

Fig. 2.

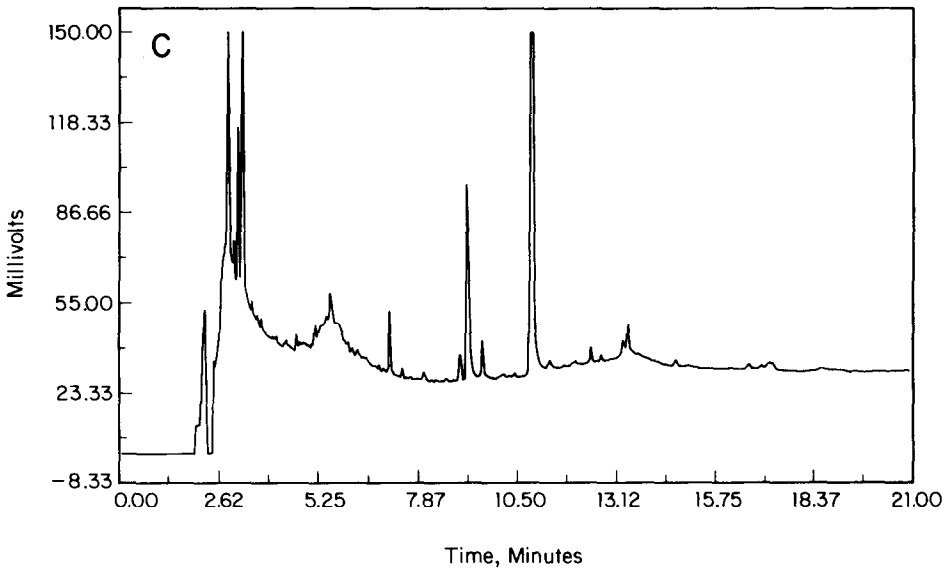

Fig. 2. Representative chromatograms of drug I, internal standard and carrier in plasma. (A) 0.5 ng/ml I, internal standard and carrier spiked in plasma; (B) clinical sample containing I (0.47 ng/ml calculated concentration), internal standard and carrier; (C) blank plasma.

added to heptane, thereby increasing polarity of the solvent, which improved extraction efficiency to 50%. In order to insure reproducible efficient extraction of the prepared plasma samples containing picogram quantities of drug, it was necessary to add carrier at the 100-ng level to minimize adsorptive loses [2]. Other liquid–liquid and solid–liquid extractions were tried resulting in either low drug recoveries or high endogenous background. High-efficiency capillary GC was employed to resolve the drug from endogenous interferences.

To achieve picogram detectability on the HP 5890 gas chromatograph, it is critical to have column exit placed within 1 mm of the collector bead, thus minimizing drug dilution with detector gases.

This method has been routinely employed for over 200 injections of plasma extracts.

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