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Note

Determination of eproxindine hydrochloride in human plasma by capillary gas chromatography

W. DAHMEN, W. GIELSDORF, B. JEREMIC, G. ACHTERT* and H.-J. HAUSLEITER

Department of Biochemistry, Kali-Chemie AG, Sparte Pharma, P.O. Box 220, D-3000 Hannover 1 (F.R.G.)

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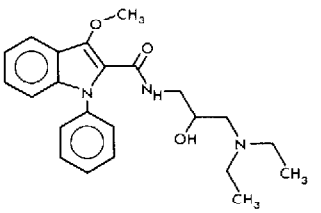
Eproxindine hydrochloride (KC 3791; I; 2-[3-(N,N-diethylamino)-2-hydroxypropylaminocarbonyl]-3-methoxy-1-phenylindole hydrochloride; Fig. 1) is a new drug that shows antiarrhythmic activity in several animal models [1–4]. The drug is in clinical phase II at the moment. Chemically the drug is not related to other drugs on the market.

This paper describes a method for the sensitive and selective quantitation of eproxindine in human plasma with the accuracy and precision needed in clinical pharmacokinetic studies.

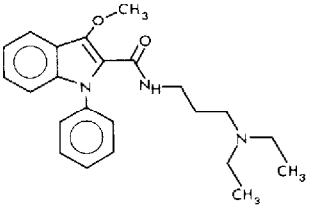
EXPERIMENTAL

Materials

Pure samples of eproxindine hydrochloride (I) and the internal standard (KC 3790; II; 2-[3-(N,N-diethylamino)propylaminocarbonyl]-3-methoxy-1-phenylindole hydrochloride; Fig. 1) were provided by Kali-Chemie (Sparte Pharma,



I



II

Fig. 1. Structures of the bases of eproxindine hydrochloride (I) and the internal standard (II).

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Hannover, F.R.G.). All other chemicals, reagents, etc. used were of analytical or reagent grade (Riedel de Häen, Seelze, F.R.G.). Samples were extracted utilizing 3-ml Extrelut® columns (E. Merck, Darmstadt, F.R.G.).

Apparatus and chromatographic conditions

A Hewlett-Packard (Palo Alto, CA, U.S.A.) 5880 A gas chromatograph equipped with a nitrogen–phosphorus detector, an automated liquid sampler 7673 A and a fused-silica, cross-linked methylsilicone capillary 19091-60312 (12 m × 0.2 mm I.D. of 0.33-μm film thickness) was used. The chromatograph was equipped with a Hewlett-Packard split injection port capillary system; the injector liner was used without additional filling material. Samples were injected in the split mode. The carrier gas was helium 4.6 [at least 99.996% purity (vol.%)] at a flow-rate of 2 ml/min. The detector purge was hydrogen 5.0 [at least 99.999% purity (vol.%)] and synthetic air at flow-rates of 2 and 80 ml/min, respectively. Gas flow-rates of make-up and split vent (1:10) were adjusted to 15 and 20 ml/min, respectively. The injector temperature was 290°C, the detector was maintained at 350°C and the oven temperature was programmed with an initial hold at 270°C for 1 min followed by an increase at 20°C/min to the final temperature of 300°C, held for 5 min.

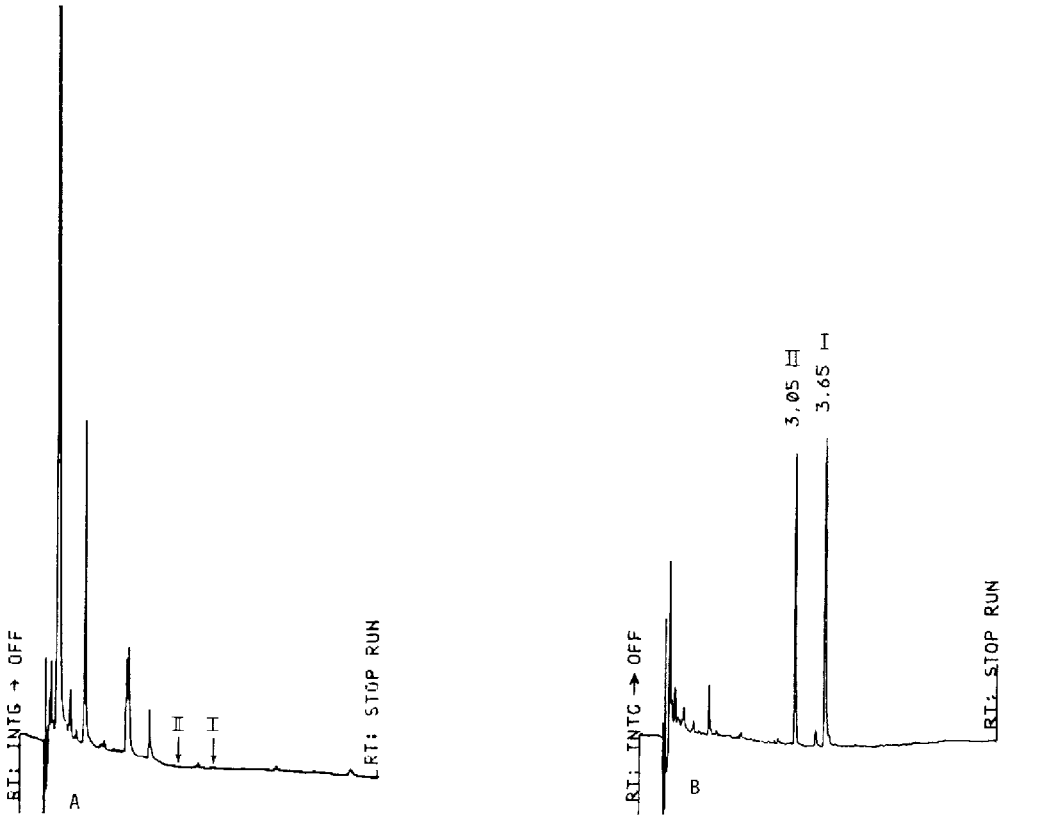

Fig. 2.

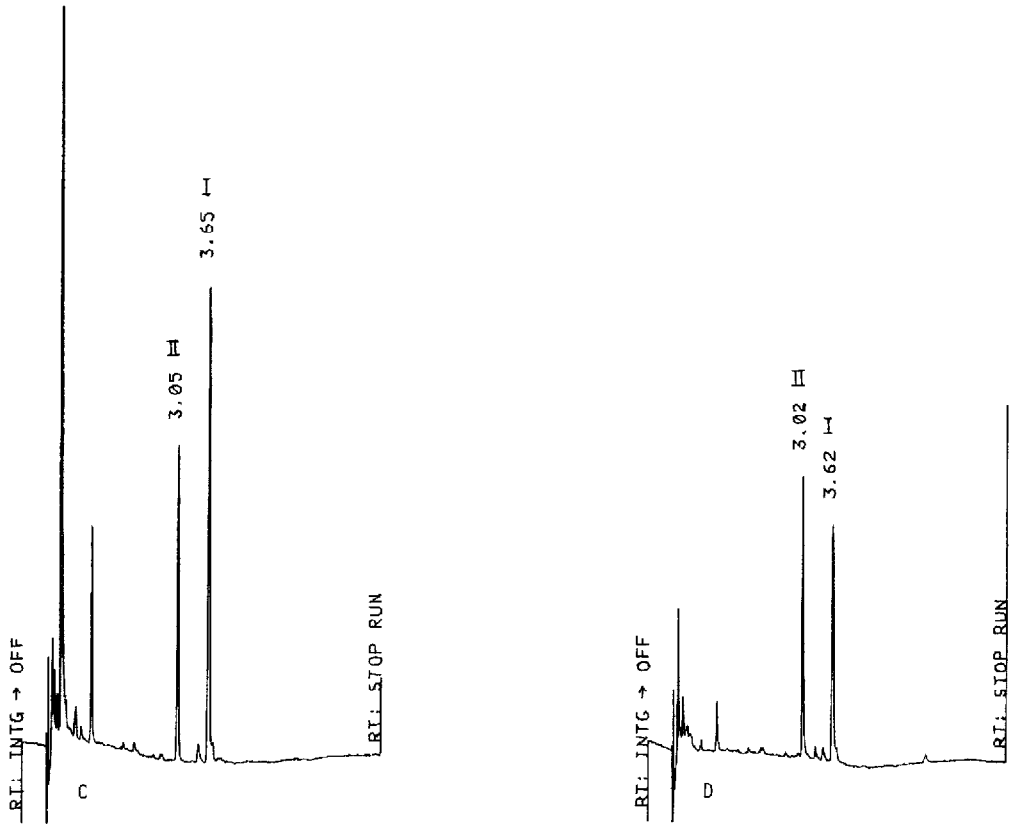

Fig. 2. Chromatograms of (A) plasma blank sample; (B) plasma spiked with 400 ng/ml II and 800 ng/ml I; (C) sample 1 h after termination of a 20-min 100-mg i.v. infusion of I (equivalent to 1250 ng/ml I); (D) sample 1 h after a 180-mg oral dose of I (equivalent to 600 ng/ml I).

Standard solutions

Stock solutions of I and II at a concentration of 100 μg/ml were prepared in ethanol. Spiking solutions containing 100, 80, 40, 20 and 10 μg/ml I and 50 μg/ml internal standard II, as well as 1 + 9 and 1 + 99 dilutions thereof in methanol were prepared by diluting the stock solutions.

Preparation of spiked samples

A 200-μl volume of an appropriate spiking solution was pipetted into a 10-ml conical glass tube, and 2 ml of plasma were added. After mixing for 10 s (Mixomat®, Boskamp, Hersel, F.R.G.), 200 μl of an ammonia solution (25%) were added. Following further mixing, the resulting sample was transferred to a 3-ml Extrelut column without preconditioning using a 2-ml pipette. Following a diffusion time of 10–15 min, the emptied sample tube was washed with 3 ml of diethyl ether. The washing solution was transferred to the column, followed by an additional elution with 10 ml of diethyl ether. The eluate was evaporated to dryness at 45 °C under a gentle stream of nitrogen. The dry residue was dissolved in 100 μl of ethanol, and 2 μl were injected.

RESULTS AND DISCUSSION

Under the chromatographic conditions employed, compound I and the internal standard II are well separated with retention times of 3.65 and 3.05 min, respectively. Elution of endogenous compounds interfering with the peaks of interest does not occur. Typical chromatograms of blank plasma free of interfering peaks, plasma spiked with I (800 ng/ml) and II (400 ng/ml) and plasma samples obtained from young healthy male subjects 1 h after oral dosing of 180 mg and 1 h after intravenous (i.v.) administration of 100 mg of I, equivalent to 600 and 1250 ng I per ml plasma, respectively, are depicted in Fig. 2A–D.

Linearity and sensitivity

For both compounds the linearity of the detector was checked first with ethanolic solutions, then with plasma blank samples (plasma was extracted and thereafter spiked; these samples were also used for the recovery experiments) and finally with spiked samples. All experiments revealed a linearity for both analytes in the concentration range 10–1000 ng/ml in plasma. The data listed in Table I indicate a lower limit of detection of 10 ng/ml in plasma.

Stability in plasma

Stability testing of different plasma samples spiked with 250 ng/ml I and analysed monthly for six months indicated that the drug is stable in plasma frozen below $-15\,^{\circ}C$ for at least six months.

Precision (reproducibility) and accuracy

The accuracy and precision of the method were evaluated by analysing six times each of the six spiked concentrations. As outlined in Table I, the assay provides results that are reliable over the range 10–1000 ng/ml, with the coefficient of variation (C.V.) never exceeding 7% and the accuracy always between +11% and −9%.

TABLE I

PRECISION AND ACCURACY EVALUATION OF THE ASSAY FOR EPROXINDINE HYDROCHLORIDE

Concentration spiked (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	*n*	C.V. (%)	Inaccuracy (%)
10	11.08 ± 0.77	5	6.97	+10.82
50	45.78 ± 1.75	6	3.83	−8.55
100	96.27 ± 4.35	6	4.52	−3.73
250	235.32 ± 13.41	6	5.70	−5.87
500	511.54 ± 18.66	6	3.65	+2.31
1000	998.04 ± 18.55	6	1.86	−0.20

Recovery

The recoveries of I and II were determined at 100 and 1000 ng/ml. Each concentration was assayed six times: the mean recoveries for I were 80 ± 6% at 1000 ng/ml and 84 ± 6% at 100 ng/ml, for the internal standard comparable values were obtained (80 ± 7% at 1000 ng/ml and 78 ± 4% at 100 ng/ml).

Calculations were made by comparing the individual data of a series of six analyses of extracted standards with the data of blank samples spiked after extraction and prior to assay.

CONCLUSION

The assay presented is well suited for a reliable, simple and fast analysis of I in human plasma samples. Samples obtained during several pharmacokinetic studies following i.v. and oral dosing have been analysed. Tests on rat and dog plasma samples indicate that it would be feasible to extend the procedure to these species.

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