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Note

Rapid gas—liquid chromatographic estimation of doxapram in plasma

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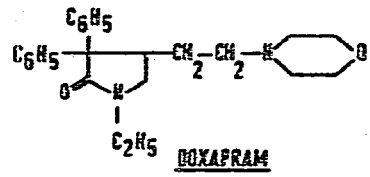
Doxapram (1-ethyl-4-(2-morpholinoethyl)-3,3-diphenyl-2-pyrrolidinone) is a respiratory stimulant which has been in clinical use for about ten years. Very little is known of its pharmacokinetics in man. Previously described methods for its estimation include high-pressure ion-exchange and thin-layer chromatography for urine [1] and UV absorption after oxidation to benzophenone for plasma [2].

We have developed a simple and sensitive gas—liquid chromatographic method for the direct estimation of doxapram in plasma. By the use of a nitrogen sensitive flame ionisation detector, only a single stage extraction is necessary and interference from the solvent front and extraneous peaks is minimal.

EXPERIMENTAL

One millilitre of 0.2 *M* borate buffer pH 9.5 was added to 2 ml of plasma in a 15-ml round-bottomed centrifuge tube and extracted with 5 ml of redistilled dichloromethane containing 0.5 μg/ml of naftidrofuryl oxalate as the internal standard. A plasma standard containing 1 μg/ml of doxapram hydrochloride was run with each set of unknown samples. After centrifugation the aqueous layer was removed by aspiration and the organic layer evaporated at 55° in a stream of air. The residue was dissolved in 20 μl of ethanol using a vortex mixer and 3-μl aliquots were injected into the gas chromatograph (Hewlett-Packard Model 5750 with 15160B nitrogen detector). The column was glass (4 ft. × 0.25 in. O.D.) packed with 1% OV-17 on Gas-Chrom Q, 80—100 mesh. The carrier gas (helium), hydrogen and air flow-rates were 60, 28 and 180 ml/min



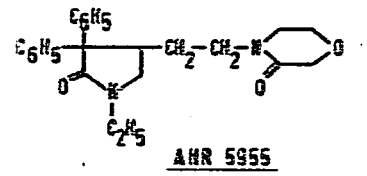


Fig. 1. Structural formulas of doxapram (1-ethyl-4-(2-morpholinoethyl)-3,3-diphenyl-2-pyrrolidinone) and AHR 5955 (1-ethyl-4-[2-(morpholin-2-one)-ethyl]-3,3-diphenyl-2-pyrrolidinone).

and the injection port, column oven and detector temperatures were 320°, 265° and 375°, respectively. The rubidium bromide crystal of the nitrogen detector was adjusted to give the maximum ionisation current.

RESULTS AND DISCUSSION

Under these conditions, the retention times of internal standard and doxapram were 2.3 min and 3.6 min, respectively, and the limit of detection was about 0.01 μg/ml. The calibration graph obtained by plotting the peak-height ratios of doxapram to naftidrofuryl versus plasma concentration of doxapram was linear up to 5 μg/ml and passed through the origin. The mean coefficient of variation for replicate analyses of doxapram added to plasma over the concentration range of 1—5 μg/ml was 2.3%. None of the known metabolites of doxapram interfered with the assay, but AHR 5955 (Fig. 1) gave a symmetrical peak and could be estimated simultaneously by temperature programming from 265° to 290° at 30°/min after an initial delay of 4 min. The calibration graph for this metabolite was also linear over the range 0.25—5 μg/ml and the coefficient of variation of replicate analyses was about 8%. Chromatograms of extracts of blank plasma and plasma obtained from a patient receiving an infusion of doxapram (4.2 mg/min) are shown in Fig. 2.

ACKNOWLEDGEMENTS

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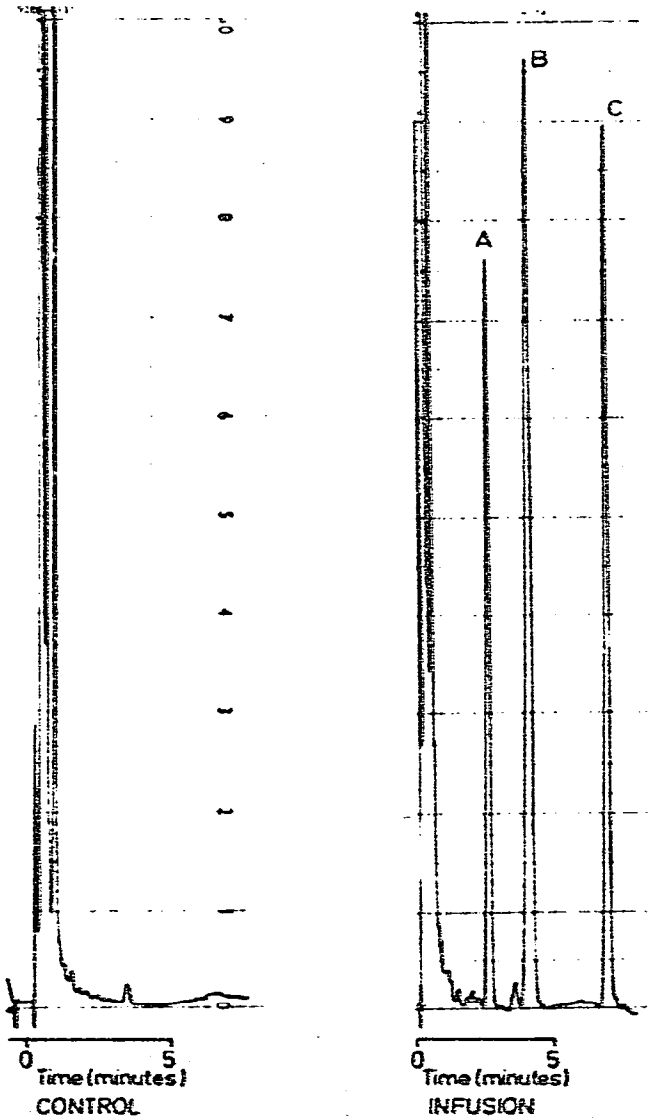

Fig. 2. Gas chromatogram of plasma extracts before, and following an intravenous infusion of doxapram (4.2 mg/min). A, Internal standard; B, doxapram (1.2 μg/ml); C, AHR 5955 (1.25 μg/ml).

REFERENCES

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