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Note

Determination of minaxolone (CCI 12923) by gas chromatography with nitrogen-sensitive detection

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Minaxolone (Mx) is a new water-soluble steroid intravenous anaesthetic which has undergone early clinical trials in different centres [1—3]. A sensitive procedure for its estimation in plasma was required for its pharmacokinetic studies. This paper describes a procedure for the estimation of Mx in the plasma of anaesthetized patients.

MATERIALS AND METHODS

Reagents were of analytical grade and solvents had been distilled in glass. All glassware was silylated, rinsed with methanol and dried. Plasma standard, 1000 ng ml^{-1} was prepared by diluting 0.5 ml of 1 mg ml^{-1} methanolic solution of Mx base with drug free plasma. Plasma standards of 800, 400, 200, 100 and 50 ng ml^{-1} of Mx were prepared by serial dilutions. Plasma standards were divided into 2-ml aliquots and frozen.

Working internal standards, 500 ng ml^{-1} of N-7084 were prepared by diluting a methanolic stock solution of 1 mg ml^{-1} of N-7084 with 2 *N* hydrochloric acid containing 5 mg ml^{-1} of trimethylamine hydrochloride.

Blood was collected from patients anaesthetized with intravenous Mx by a heparinized line inserted into the opposite arm. Plasma was stored in plastic test tubes and kept frozen until analyzed. Standards and test samples were thawed at room temperature. To 1 ml of each specimen in PTFE-lined screw-capped culture tubes (16 × 100 mm), 1 ml of working internal standard and 6—7 ml of pentane were added. The contents of the tubes were mixed on a rotary mixer for 10 min. The tubes were centrifuged and the upper layer discarded by suction. To each tube, 1 ml of 2.5 *N* sodium hydroxide solution and 6—7 ml of pentane were added. The tubes were shaken on a rotary mixer for

10 min. The upper pentane layer was collected into tubes, taking care not to collect any emulsion or aqueous droplets. The aqueous phase was once again extracted with 6—7 ml of pentane and the upper layer collected. The combined pentane layers were evaporated to dryness at 45—50°C. The residue in each tube was dissolved in 20 μl of methanol by vortex mixing. The tubes were kept well stoppered until their contents (1—2 μl aliquots) were analyzed by gas chromatography (GC).

A Varian 3700 gas chromatograph (Walnut Creek, CA, U.S.A.) equipped with a thermionic nitrogen—phosphorus detector and a glass column (1.8 m × 4 mm ID) packed with 3% OV-17 on Gas-Chrom Q (100—120 mesh) was used. The oven, injector, and detector temperatures were 265, 280, and 300°C, respectively. The carrier gas was nitrogen at a flow-rate of 30 ml min^{-1}. The chromatogram was recorded with a Varian recorder and the areas of the peaks were computed with an Autolab System I integrator (Spectra Physics, Santa Clara, CA, U.S.A.).

RESULTS AND DISCUSSION

In the last few years, many drugs present in blood at ng ml^{-1} level have been analyzed by GC with the use of nitrogen-selective detectors. With the improvements in the design of these detectors, it has become relatively easy to use them [4]. Since Mx has a tertiary amino group, we elected to use a nitrogen-selective detector for higher sensitivity and selectivity for the assay of Mx. Some brands of blood-collecting devices contain phosphorus compounds as lubricants which produce potentially interfering peaks when a nitrogen-selective detector is used. Devices free from such contaminants were used for collecting blood for this study. After trying a number of tertiary amines, N-7084 (Fig. 1) was found to be a suitable internal standard. As seen in Fig. 2, it is well separated from the solvent peak and from the Mx peak. Minor tranquilizers (e.g. diazepam), which are frequently administered prior to surgery did not interfere with either the Mx peak or with the internal standard peak. After the addition of internal standard, plasma is washed at a pH of about 2 with pentane to remove some of the neutral or acidic impurities. Mx and the internal standard are not extracted by pentane at this pH. After adjusting the pH to greater than 10, plasma is extracted twice with pentane. Pentane is a non-polar solvent and extracts endogenous impurities in poor yield. The uncorrected recovery of

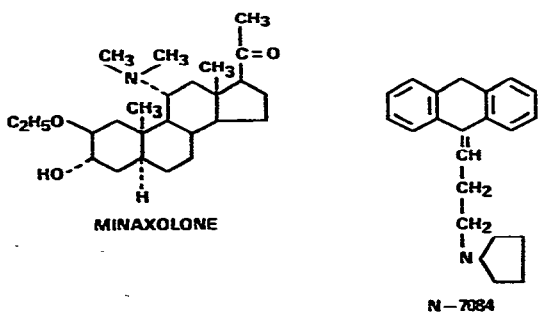

Fig. 1. Structural formulae of minaxolone (Mx) and N-7084.

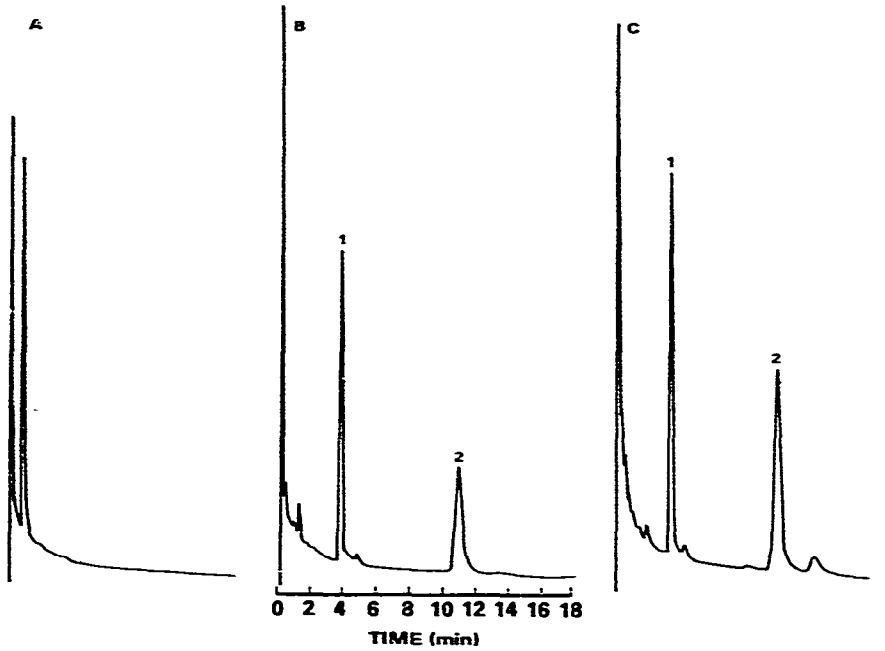

Fig. 2. Chromatograms of extracts of plasma (attenuation 32 × 10^{-12}). (A) Extract of drug-free pooled plasma; (B) extract with added Mx (200 ng/ml); (C) extract of plasma of a patient anaesthetized with Mx (320 ng/ml). Peaks: 1, N-7084 (internal standard); 2, Mx.

Mx is about 55% as determined by analyzing supplemented plasma without the addition of internal standard and comparing the peak areas with those obtained by injecting the same aliquots of non-extracted standards. The losses due to absorption during extraction are minimized by silylating the glassware and by addition of trimethylamine as a scavenger. During evaporation of the extract, trimethylamine is completely removed. Mx also has a secondary alcoholic group. Alcohols are polar compounds and have a tendency to adsorption on columns during GC analysis. However, the peak of Mx is sharp and symmetrical (Fig. 2) and the standard curve is linear for the range tested (50—1000 ng ml^{-1}) either by comparing the ratio of peak areas obtained by electronic integration or by comparing the ratios of peak heights for Mx and internal standard. The calibration curve passes through the origin. Haloperidol which has similar functional groups (a tertiary amino group, secondary alcoholic group and a keto group) has been analyzed in plasma in very low concentration by GC using a nitrogen-selective detector without any derivatization [5].

The coefficients of variation obtained by analyzing 10 replicate 50, 200, and 800 ng ml^{-1} plasma standards yielded values of 9.7, 4.6 and 9.8%, respectively. Prepared plasma solutions of Mx were found to remain unchanged after storage at —15°C for three months by extraction and comparison of concentrations of a similar set of freshly prepared samples.

This procedure was used to study the pharmacokinetics of Mx. An example of the plasma concentration curve for a female patient after receiving a bolus of 62.5 mg of Mx intravenously is shown in Fig. 3. This procedure allowed us to delineate plasma Mx concentration as low as 30 ng ml^{-1}.

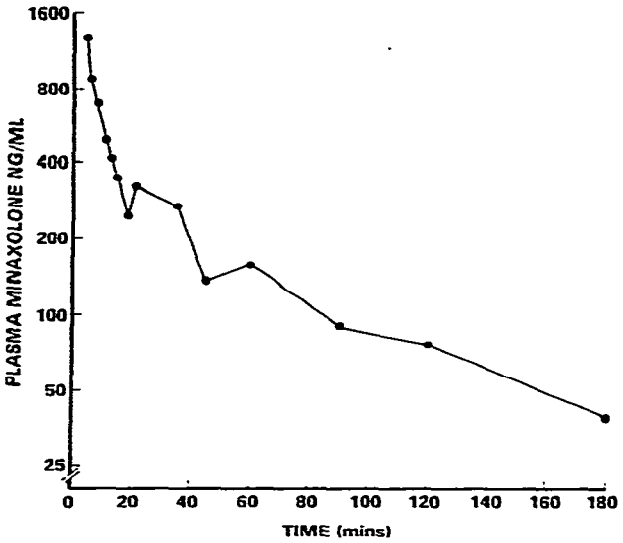

Fig. 3. Plasma profile of a female patient after administration of 62.5 mg of Mx intravenously.

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