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Note

Determination of nitromethaqualone in blood by electron-capture—gas chromatography

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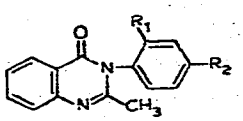
Nitromethaqualone (I), the active compound from Parnox^R, or 2-methyl-3-(2′-methoxy-4′-nitrophenyl)-4(3H)-quinazolinone is a non-barbituric hypnotic that has been used in Europe since 1967. Methaqualone (III), the active compound from Cateudyl^R, Mequalone^R, Mollinox^R, Noxybel^R, Revonal^R, Toquilone^R, and mecloqualone (IV), the active compound from Nubarène^R, are two other products of the quinazolinone series. Numerous papers describe the gas chromatographic analysis of methaqualone and mecloqualone in biological samples [1--7]. No procedure for the analysis of nitromethaqualone in biological samples has been described. The gas chromatographic method described by Daenens and Van Boven [8] for the identification of quinazolinones in illicit mixtures is not sensitive enough when applied to biological samples. The present paper describes the identification of nitromethaqualone in blood by gas—liquid chromatography with electron-capture detection after solvent extraction of the drug along with the previously added internal standard.



R_1	R_2		
OCH_3	NO_2	I	nitromethaqualone
H	NO_2	II	internal standard
CH_3	H	III	methaqualone
Cl	H	IV	mecloqualone

MATERIALS AND METHODS

Materials

The extraction solvents were of analytical grade. Nitromethaqualone for establishing the calibration graph was prepared by extraction of Parnox^{R} tablets [8] followed by careful recrystalisation (m.p. 193°C). The internal standard [2-methyl-3-(4′-nitrophenyl)-4(3H)-quinazolinone] was synthesized according to Grimmel et al. [9]. White crystals were obtained, m.p. 197°C.

Apparatus

A Becker 420 gas chromatograph, equipped with a $3.7 \cdot 10^8$ Bq ^{63}Ni pulse-frequency-modulated electron-capture detector was used; pulse period was 200 μsec, pulse width 0.5 μsec. The chromatograph was operated with a glass column (1.5 m × 2 mm I.D.), inactivated with dimethylchlorosilane in toluene and packed with 2% OV-17 on Chromosorb AW (100—120 mesh). The carrier gas was dry nitrogen at a flow-rate of 50 ml/min. The instrument settings were as follows: temperature of the oven 255°C, temperature of both injector and detector 280°C.

Assay of plasma samples

To 1.0 ml of human blood in a centrifuge tube were added 2.0 ml ammonia buffer (pH 9.2) followed by 0.5 ml of internal standard solution (0.01 mg% in water) and 1 ml of the extracting solvent consisting of a mixture of toluene—hexane—isoamyl alcohol (78:20:1). The blood was extracted for 20 min by means of a mechanical rotator. The layers were separated by centrifuging for 3 min at 1100 *g* and 1-μl aliquots of the organic phase used for injection into the gas chromatograph.

Quantitation

The concentrations of nitromethaqualone in blood were calculated with the aid of calibration graphs, which were prepared as follows. Methanolic solutions containing 10, 20, 40, 60, 80, 110, 140 and 200 ng of nitromethaqualone were evaporated and the residue dissolved in 1.0 ml of blood. The samples prepared were analysed by the procedure described above. The ratios of the peak heights of nitromethaqualone to that of the internal standard were plotted against the known concentrations of nitromethaqualone.

Recovery studies

We measured the absolute recovery of nitromethaqualone from blood and the internal standard as follows. Solutions of 25 and 50 ng of nitromethaqualone and internal standard, respectively in 1.0 ml of the extracting solvent were prepared. Carefully measured 2-μl aliquots of these solutions were chromatographed and the peak heights determined. To the prepared solutions were added 1 ml of blood, 2 ml of buffer and 0.5 ml of water. After mixing with a mechanical rotator for 20 min, the layers were separated by centrifuging. Again exactly 2-μl aliquots were injected in the gas chromatograph and the peak heights determined. Percentage recovery was calculated by comparing these peak heights with the peak height obtained by the injection of solution of the pure compound.

RESULTS AND DISCUSSION

The greater sensitivity of the electron-capture detector for nitromethaqualone along with the use of an internal standard makes the quantitative analysis of nitromethaqualone in blood relatively simple. Concentrations down to 1 ng/ml of blood can still be measured. The procedure was shown to be specific for nitromethaqualone as no interfering peaks from constitutents of normal blood in the same region as either nitromethaqualone or internal standard have been found. Fig. 1 shows typical gas chromatograms of an extract of a drug-free blood, an extract from a 1.0-ml blood sample to which 80 ng nitromethaqualone were previously added and an extract from a 1.0-ml blood sample collected from a volunteer after oral intake of 25 mg nitromethaqualone. According to the calibration graph there is a linear relation between the ratio of the peak height of nitromethaqualone to that of the internal standard and the concentration of nitromethaqualone up to 100 ng/ml of blood when 1-μl aliquots were injected. The concentrations of nitromethaqualone in blood samples can be read directly from the previously constructed calibration graph (Fig. 2).

Extraction yields for one single extraction are 99.1 ± 2% for both nitromethaqualone and the internal standard.

The good resolution from both the solvent peak and nitromethaqualone makes 2-methyl-3-(4′-nitrophenyl)-4(3H)-quinazolinone (II) a good choice as internal standard for nitromethaqualone assays.

The procedure has been applied to measure plasma concentrations of two volunteers after oral intake of 25 mg nitromethaqualone hydrochloride. Peak plasma levels of 65 and 135 ng/ml were observed after 120 and 90 min respectively. Fig. 3 shows blood concentration graphs after oral intake of 25 mg nitromethaqualone hydrochloride by those two volunteers.

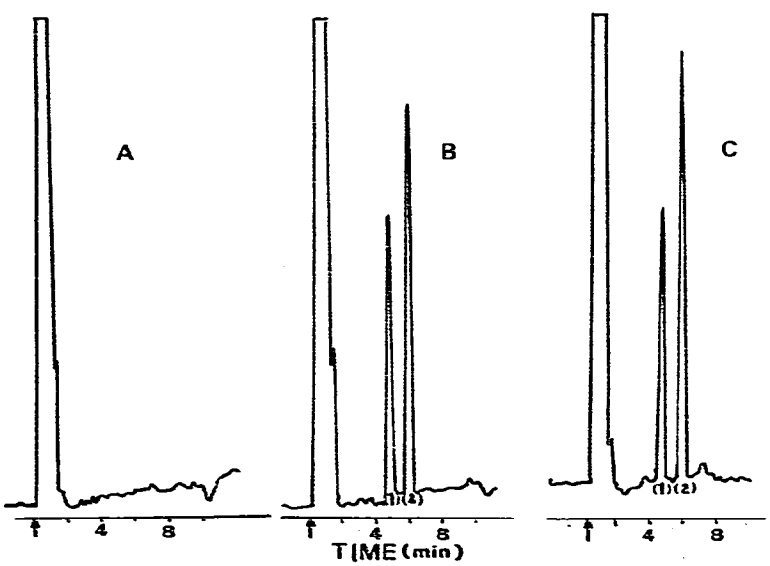


Fig. 1. Typical gas chromatograms of blood extracts from (A) drug-free blood without addition of internal standard; (B) blood to which 80 ng nitromethaqualone and 50 ng internal standard were added and (C) blood sample from a volunteer after intake of 25 mg nitromethaqualone. Peaks: 1 = internal standard, 2 = nitromethaqualone.

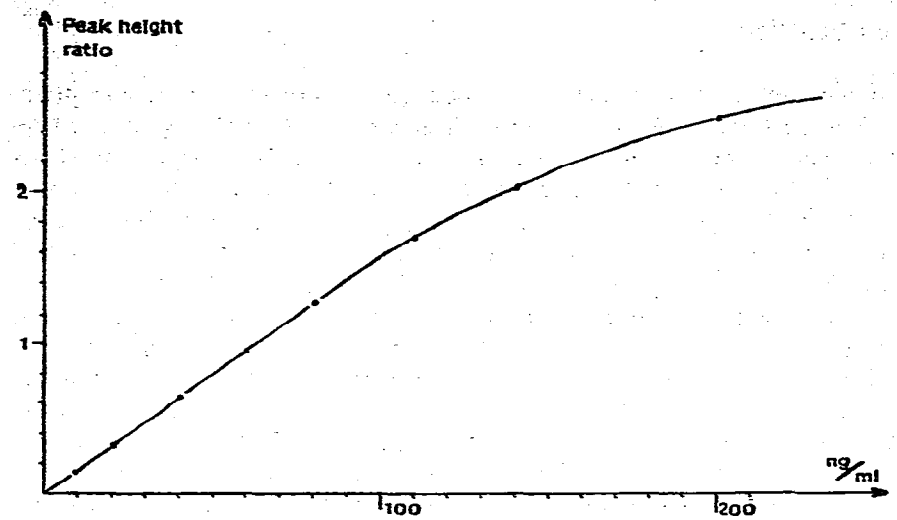


Fig. 2. Calibration graph for the determination of nitromethaqualone in blood.

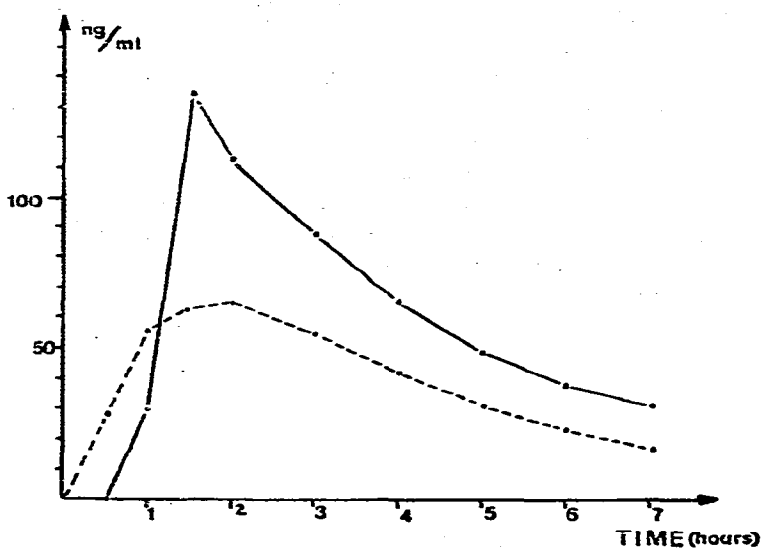


Fig. 3. Blood concentration graphs from two volunteers after oral intake of 25 mg nitromethaqualone hydrochloride.

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