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GAS CHROMATOGRAPHIC DETERMINATION OF METHAQUALONE, 2-METHYL-3-*o*-TOLYL-4(3H)-QUINAZOLINONE, AT THERAPEUTIC LEVELS IN HUMAN PLASMA

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SUMMARY

A specific and sensitive procedure for the determination of methaqualone at therapeutic levels in human plasma has been developed. The method involves extraction of the drug from alkalinised plasma into hexane, with subsequent separation on a gas–liquid chromatograph fitted with a flame ionisation detector. Butobarbitone is used as an internal standard for quantitation by the relative peak height technique. Plasma levels encountered after oral ingestion of a single therapeutic dose are reported.

INTRODUCTION

The non-barbiturate hypnotic, methaqualone, is widely prescribed in the hydrochloride form as "Melsedin" and also, increasingly, in combination with diphenhydramine as "Mandrax". Spectrophotometric methods for the analysis of methaqualone in plasma have been described[1,2], but these seem to be insensitive in the concentration range encountered after the recommended therapeutic dosage. Further, it has been our experience that the high degree of background interference, which frequently occurs when applying these procedures, can render an accurate determination of plasma concentration difficult, even in some cases of overdosage.

NANIKAWA AND KOTOKU[3] and BOGAN[4] have described the gas chromatographic separation of methaqualone on an SE-30 column. LEACH AND TOSELAND[5] have shown that the drug will also chromatograph on a 10% Apiezon L column. However, no quantitative measurement of the drug in biological fluid by gas chromatography has been reported.

The present paper describes the application of gas–liquid chromatography (GLC) to the determination of methaqualone in biological fluids, after preliminary separation by solvent extraction.

EXPERIMENTAL

Reagents

The following reagents were used: hexane fraction of petroleum spirit (Hopkin

& Williams Ltd., Chadwell Heath, Essex); 1 *N* sodium hydroxide and 0.1 *N* hydrochloric acid (reagent grade) purified by ether washing; anhydrous sodium sulphate purified by heating at 650° for 24 h prior to use. The internal standard was a 2 mg% solution of butobarbitone (May & Baker Ltd., Dagenham, Essex) in ether.

Gas chromatography

A Pye 104 model 24 dual column gas chromatograph, equipped with a flame ionisation detector and a 1 mV Honeywell recorder, was used. The column was a 7 ft. × ¼ in. internal diameter coiled glass-tube, which had been silanised with a 5% solution of dimethyldichlorosilane in benzene over a period of 24 h. Glass-wool was silanised in the same solution. After drying at 100°, the column was packed with 3% cyclohexanedimethanol succinate (CDMS) (Perkin-Elmer Ltd., Beaconsfield, Bucks.) on 85–100 mesh Diatomite CQ (J.J.'s Chromatography Ltd., King's Lynn, Norfolk). This packing was prepared as follows: 0.77 g of CDMS were dissolved in 100 ml of dichloromethane. The support (24 g) was added to the flask and left to stand, with occasional swirling, for 2 h. The solvent was removed under vacuum in a rotary evaporator, the final stages of evaporation being completed in a water-bath at 100° for 30 min. The prepared column was then packed with the coated support by closing one end with silanised glass-wool and applying a vacuum. After filling the other end was closed with silanised glass-wool and the packed column conditioned for 48 h at 250° with a nitrogen flow rate of 55 ml/min. At the end of this time the column was tapped down, more coated support added and conditioning for a further 24 h carried out. This column has been in constant use for 18 months and no significant change in behaviour has been observed. The instrument settings were as follows: temperature, column 200°, injection port 240°; gas flow rates, hydrogen 45 ml/min, nitrogen (carrier gas) 55 ml/min, air 400 ml/min; sensitivity, 2×10^{-10} A.

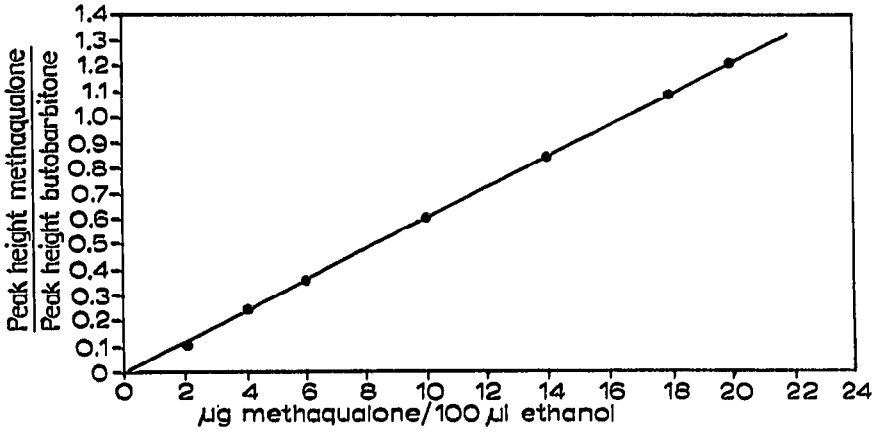
Extraction procedure

A sample of 5.0 ml of plasma was made alkaline by the addition of 1.0 ml of 1 *N* sodium hydroxide, and extracted with 15 ml of hexane by gentle shaking for 10 min in a 30-ml centrifuge-tube. After centrifugation at 3,000 r.p.m., the organic layer was transferred to a second tube containing approximately 3 g of anhydrous sodium sulphate. On carrying out a second, 5 min extraction with 10 ml of hexane, the organic fractions were bulked, thoroughly shaken with the anhydrous sodium sulphate, and left to stand for 10 min. The extract was then evaporated to dryness under a stream of nitrogen in a 10-ml conical centrifuge-tube to which 1.0 ml of the butobarbitone standard solution had been added, the tube being immersed in a water-bath at 60°. The residue was taken up in 100 μl of absolute ethanol, and 3–5 μl of this were injected on to the gas chromatograph.

RESULTS AND DISCUSSION

Quantitation

Butobarbitone was chosen as an internal standard, since the column used will separate barbiturates, and this analogue has good resolution from both the solvent peak and the methaqualone peak. Furthermore, the acidic barbiturates will be excluded from the alkaline extract. A range of standard solutions, containing 200 μg/ml



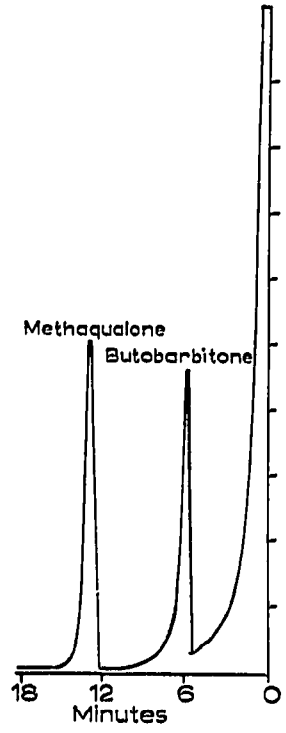
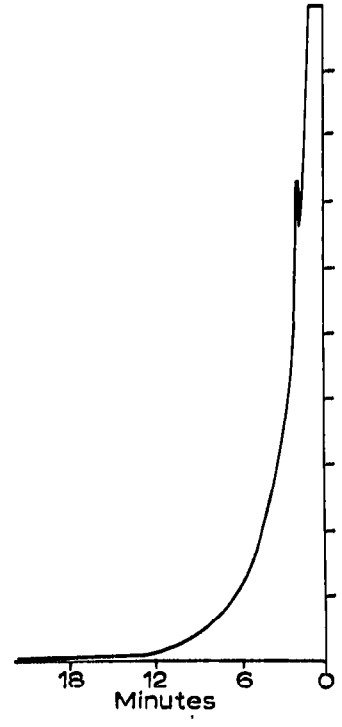


Fig. 1. The standard calibration graph relating the ratio of the peak heights of methaqualone and butobarbitone to the number of micrograms of methaqualone in the extract.

Fig. 2. Standard methaqualone solution. The separation of methaqualone and the butobarbitone internal standard.



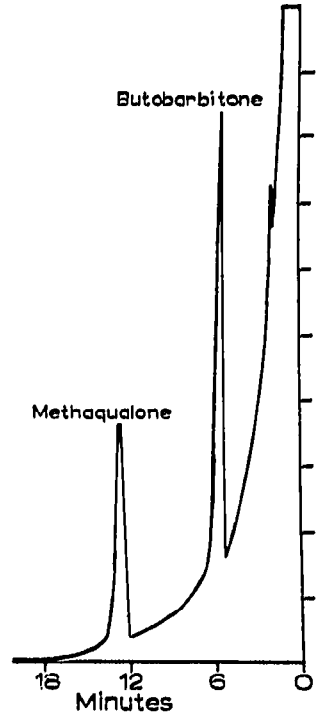


Fig. 3. Gas chromatogram of a hexane extract of human plasma. Chromatogram from normal plasma without addition of butobarbitone.

Fig. 4. Gas chromatogram of a hexane extract of human plasma. Chromatogram of an extract from an individual treated with methaqualone.

of butobarbitone and from 20 μg to 200 μg/ml of methaqualone, were made in ethanol. A standard curve was prepared by injecting 3–5 μl aliquots of these solutions just prior to measuring unknown samples. The ratio of the peak height of methaqualone to butobarbitone was linear over the range 0.1 μg to 1 μg of methaqualone on injection

TABLE I

DRUGS FOUND NOT TO INTERFERE WITH GLC METHOD FOR MEASUREMENT OF METHAQUALONE

Valium	Diphenhydramine hydrochloride
Chloral hydrate	Propoxyphene hydrochloride
Nitrazepam	Imipramine hydrochloride
Glutethimide	Amitriptyline hydrochloride
Meprobamate	Chlorpromazine hydrochloride
Methyprylon	Diphenylhydantoin

(Fig. 1). The relative retention time of methaqualone with respect to butobarbitone was 2.14 (Fig. 2).

Recovery studies

Previous studies on the extraction of methaqualone from biological material have shown that the drug is quantitatively extracted into hexane[2]. Amounts ranging from 2 to 20 μg of methaqualone dissolved in 0.1 *N* hydrochloric acid were added to 5 ml of plasma to verify the adequacy of the extraction procedure. In nine experiments, the mean recovery achieved was 96% with a standard deviation of 3.3.

TABLE II

METHAQUALONE PLASMA LEVELS (mg/100 ml) IN FIVE SUBJECTS FOLLOWING A SINGLE ORAL DOSE

Subject	1	2	3	4	5
Sex	M	M	F	M	F
Age (yr.)	23	32	22	23	43
Weight (lb.)	144	160	108	146	147
Dose (mg)	150	150	150	250	250
Time (h)					
0	0.0	0.0	0.0	0.0	0.0
0.5	0.079	0.0	0.001	0.220	0.208
1.0	0.099	0.023	0.226	0.220	0.224
1.5	0.102	0.145	0.150	0.210	0.191
2.0	0.113	0.105	0.115	0.190	0.186
2.5	0.096	0.074	0.092	0.180	0.159
3.0	0.077	0.050	0.080	0.170	—
3.25	—	—	—	—	0.127
3.5	0.077	0.054	0.066	0.150	—
4.0	0.062	0.042	—	0.140	0.096
4.25	—	—	0.050	- -	—
5.0	0.046	0.034	—	--	—
5.25	—	—	0.046	—	—
5.5	—	—	—	0.110	0.073
6.0	—	0.027	—	—	—
6.5	—	—	0.043	0.110	0.058
7.0	0.043	—	—	—	—
7.25	—	—	0.042	—	—
7.5	—	0.020	—	0.090	0.055

TABLE III

URINARY OUTPUT OF FREE METHAQUALONE DURING THE EIGHT HOURS FOLLOWING AN ORAL DOSE

Subject No.	*Dose (mg)*	*Methaqualone excreted (mg)*
1	150	0.042
2	150	0.020
4	250	0.210
5	250	0.213

Specificity

The procedure has been shown to be specific for methaqualone. No interfering peaks in the same region as either methaqualone or butobarbitone have been encountered from constituents of normal plasma and urine (Figs. 3, 4), or from exogenous components in samples derived from patients receiving a variety of other drugs (Table I).

Application

The procedure has been used to measure plasma and urine levels of methaqualone in human subjects, following the administration of a single oral dose of 150 mg (Melsedin) and 250 mg (Mandrax) in tablet form. The results from five experiments are tabulated (Tables II and III). Fig. 5 illustrates that in one subject the plasma level reached a maximum of 0.226 mg% 1 h after ingestion and the drug could still be measured 7 h later. The red blood cells from some of the experiments have also been analysed. After washing with isotonic saline, they were haemolysed with cold water before extraction. In human subjects following the ingestion of a single Mandrax tablet, the distribution of methaqualone between the red cells and plasma was found to be 6% and 94% respectively. Previous research[6] has shown that in rat blood the distribution is 26% and 74%. Only a small amount of free methaqualone is found in the urine after therapeutic administration (Table III), but considerably greater amounts are found in cases of overdosage. The method has also been applied to the analysis of dialysis

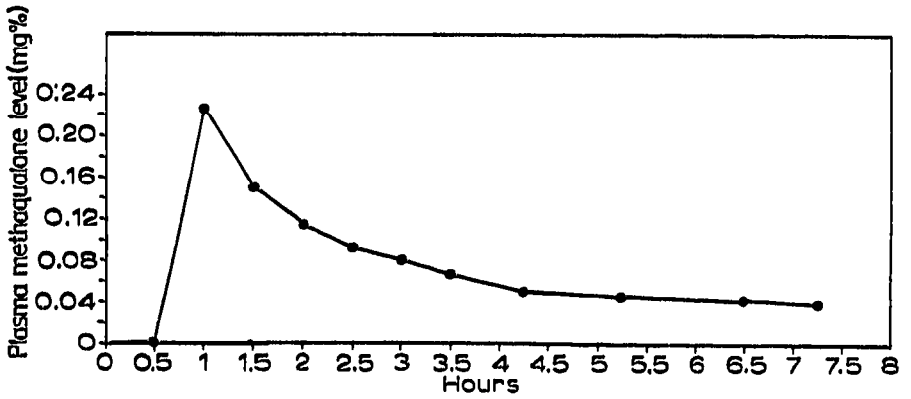


Fig. 5. A typical plasma methaqualone concentration/time curve indicating the levels found in a subject following an oral dose of 150 mg of methaqualone.

fluid from overdose subjects in order to examine the efficacy of this treatment in such cases. Further investigations are required, however, before definite conclusions can be drawn from these studies.

No attempt has been made at a comparative study between the spectrophotometric and gas chromatographic methods of analysis. It is envisaged, however, that the application of this latter procedure to cases of methaqualone overdosage will serve to clarify some of the discrepancies in published plasma levels.

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