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AN IMPROVED QUANTITATIVE GAS-LIQUID CHROMATOGRAPHIC ASSAY FOR THE ESTIMATION OF METHAQUALONE IN BIOLOGICAL FLUIDS

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SUMMARY

An improved, sensitive and specific method has been developed for the estimation of methaqualone in human biological fluids, after therapeutic doses. The procedure involves preliminary solvent extraction of methaqualone from alkaline body fluids into diethyl ether, followed by concentration and estimation on a gas-liquid chromatograph fitted with a flame ionization detector. Methadone hydrochloride is used as the internal marker standard for quantitation of the procedure by the peak-height ratio technique. The method can be used to determine down to 0.02 μg of methaqualone per millilitre of sample, and has been applied to the measurement of methaqualone concentrations found in human plasma, erythrocytes and urine after single, oral therapeutic doses.

INTRODUCTION

Methaqualone was introduced in 1958 as a rapid, orally active, non-barbiturate hypnotic. BOISSIER *et al.*¹ confirmed its effect on the central nervous system and the clinical uses have been described by RAVINA² and ARVERS³.

In Britain, methaqualone is widely prescribed in the hydrochloride form as "Melsedin" (Boots), 150 mg, and as "Mandrax" (Roussel), 250 mg with diphenylhydramine, 25 mg.

The gas chromatographic separation of methaqualone on an SE-30 column has been described by NANIKAWA AND KOTOKU⁴ and BOGAN⁵. LEACH AND TOSELAND⁶ have shown that the drug will also chromatograph on a 10% Apiezon L column. Recently, BERRY⁷ reported a quantitative gas-liquid chromatographic method, which he used successfully to measure plasma methaqualone concentrations in man from therapeutic doses of the drug. The lengthy extraction procedure into hexane required long periods of centrifugation to separate the emulsion. Quantitation was achieved by the addition of an external marker substance, butobarbitone, before evaporating to dryness under a stream of nitrogen, after which the residue was taken up into absolute ethanol before being injected into the gas chromatograph.

The present study was therefore undertaken to find a rapid gas chromatographic method of analysis with an internal marker for quantitation of results. All errors arising from the extraction procedure can be minimized by the use of the

internal marker technique and this method therefore provides a more sensitive and accurate method of analysis.

EXPERIMENTAL

Materials and apparatus

The following materials were used: analaR diethyl ether, freshly re-distilled; and sodium hydroxide solution, 20 % (w/v), containing sodium chloride, 4.5 % (w/v), purified by washing with diethyl ether. The internal markers were: methadone hydrochloride, 10 $\mu\text{g}/\text{ml}$ and 2.5 $\mu\text{g}/\text{ml}$ in distilled water.

The apparatus used were: centrifuge tubes with well fitting stoppers; stoppered evaporating tubes, capacity 15 ml, with finely tapered bases; and Perkin-Elmer F-11 gas-liquid chromatograph fitted with a flame ionization detector and a parametric amplifier, 2.5 V, linked to a Hitachi 159 recorder.

Gas chromatography

Initially, two stainless-steel columns were investigated. The first, a 1 m \times $\frac{1}{8}$ in. O.D. column of 5 % silicone gum rubber (SE-30) on Chromosorb G, 80-100 mesh, acid-washed and hexamethyldisilazane (HMDS)-treated, was conditioned for 24 h under operating conditions of oven temperature 215°, injection port temperature 225°, nitrogen (carrier gas) flow-rate 50 ml/min, hydrogen flow-rate 45 ml/min and air flow-rate 400 ml/min. The second, a 2 m \times $\frac{1}{8}$ in. O.D. column packed with 3 % cyclohexanedimethanol succinate on Diatomite CQ, 85-100 mesh, HMDS-treated, was conditioned for 24 h under operating conditions of oven temperature 250°, injection port temperature 280°, nitrogen (carrier gas) flow-rate 50 ml/min, hydrogen flow-rate 46 ml/min and air flow-rate 400 ml/min.

The following four glass columns (6 ft. \times $\frac{1}{4}$ in. O.D.) were subsequently investigated: (i) 10 % Apiezon L with 10 % potassium hydroxide on Chromosorb G, 80-100 mesh, oven temperature 250°; (ii) 2 % Carbowax 6000 with 5 % potassium hydroxide on Chromosorb G, 80-100 mesh, oven temperature 250°; (iii) 3 % cyclohexanedimethanol succinate on Diatomite CQ, 85-100 mesh, HMDS-treated, conditioned for 24 h under operating conditions of oven temperature 240°, injection port temperature 285°, nitrogen (carrier gas) flow-rate 200 ml/min, hydrogen flow-rate 54 ml/min and air flow-rate 460 ml/min; (iv) 3 % Silicone OV-17 on Chromosorb G, 60-80 mesh, acid-washed and DMCS-treated, conditioned for 24 h under operating conditions of oven temperature 240°, injection port temperature 285°, nitrogen (carrier gas) flow-rate 200 ml/min, hydrogen flow-rate 54 ml/min and air flow-rate 460 ml/min.

All columns were silanized *in situ* with two 5 ml volumes of HMDS before use.

Chlorpheniramine, diphenhydramine, pethidine, droperidol, pentazocine, methadone hydrochloride and brompheniramine maleate were investigated as possible internal markers for quantitation of the method of analysis.

Procedure. A sample of 5.0 ml of standard methaqualone solution, plasma or urine or 2.5 ml of erythrocytes lysed with 2.5 ml of water was made alkaline by the addition of 0.5 ml of 20 % (w/v) sodium hydroxide solution containing 4.5 % (w/v) sodium chloride, 1.0 ml of internal marker solution was added, and the solution was extracted with re-distilled diethyl ether (3 \times 2.5 ml) by gentle shaking for 2 min;

any emulsion formed was broken by centrifuging for 5 min at 3000 *g*. The ethereal layer was removed carefully with a pipette and placed in an evaporating tube with a finely tapered base⁸ (it is essential to avoid transfer of the aqueous phase). The pipette was rinsed with diethyl ether into the evaporating tube before proceeding with each extraction and the combined extractions were concentrated by the method described by REYNOLDS AND BECKETT⁹. The concentrated extract (2 μ l) was injected on to the column of the gas-liquid chromatograph. The concentration of methaqualone was obtained by calculating the ratio of the peak heights of methaqualone to internal marker and relating this to a previously constructed calibration graph.

Quantitation. Standard calibration graphs were prepared by using aqueous solutions of methaqualone (5 ml) containing 0.5–4.0 μ g/ml. Ratios of peak heights of methaqualone to internal marker were plotted against methaqualone concentration. The calibration factors were 3.6 and 13.7, respectively.

Recovery studies. Previous studies by AKAGI *et al.*¹⁰ and BERRY⁷ on the extraction of methaqualone from biological fluids have shown that the drug is quantitatively extracted into hexane.

Solutions of methaqualone (4 μ g/ml) in plasma or urine and 50% erythrocytes in water were used to investigate the efficiency of extraction by diethyl ether. Seven samples of each fluid (5.0 ml) were assayed and related to a 100% value obtained from a standard solution (5.0 ml) of methaqualone, 4 μ g/ml in diethyl ether, concentrated after the addition of the internal standard solution and chromatographed as described for the combined ethereal extracts.

RESULTS AND DISCUSSION

Diphenhydramine, pethidine and droperidol were found to be unsuitable as internal standards because of their very short retention times. Pentazocine was also unsuitable because it had the same retention time as methaqualone. Chlorpheniramine, brompheniramine and methadone had suitable retention times and were further investigated. Their retention times and symmetry factors, together with those obtained for methaqualone, are shown in Table I.

TABLE I
COMPARISON OF RETENTION TIMES AND SYMMETRY FACTORS

Column	Brompheniramine		Chlorpheniramine		Methaqualone		Methadone	
	Retention time (min)	Symmetry factor	Retention time (min)	Symmetry factor	Retention time (min)	Symmetry factor	Retention time (min)	Symmetry factor
<i>Stainless steel</i>								
SE-30	—	—	2.9	1.29	5.7	1.35	—	—
Cyclohexane di-methanol succinate	2.7	1.25	—	—	6.3	1.20	—	—
<i>Glass</i>								
Cyclohexane di-methanol succinate	3.64	1.75	—	—	7.32	1.16	2.56	2.0
OV-17	4.20	1.19	—	—	6.2	1.05	4.04	1.12

The OV-17 glass column was found to be the most suitable for the chromatography of methaqualone, and methadone the most suitable internal marker. Methadone is extracted efficiently by diethyl ether from alkaline biological fluids¹¹, has some similarity in structure to that of methaqualone and was well resolved both from the solvent front and the methaqualone peak, as shown in Fig. 1.

The standard calibration graph relating the peak-height ratio of methaqualone and methadone to the number of micrograms of methaqualone in the extract was linear over the range 0.01–20 μg , as shown in Fig. 2. The procedure was shown to be specific for methaqualone, as no interfering peaks in the same region as either methaqualone or methadone have been found from constituents of normal plasma or urine. During the analysis of erythrocyte samples, other peaks were sometimes present on the chromatogram, one of which interfered slightly with the methaqualone peak.

The recoveries of methaqualone from urine, plasma and erythrocytes by extracting into diethyl ether were 89.1% (S.D. = 2.0%), 88.2% (S.D. = 2.3%) and 85.3% (S.D. = 2.50%), respectively. BERRY⁷ reported the mean recovery of

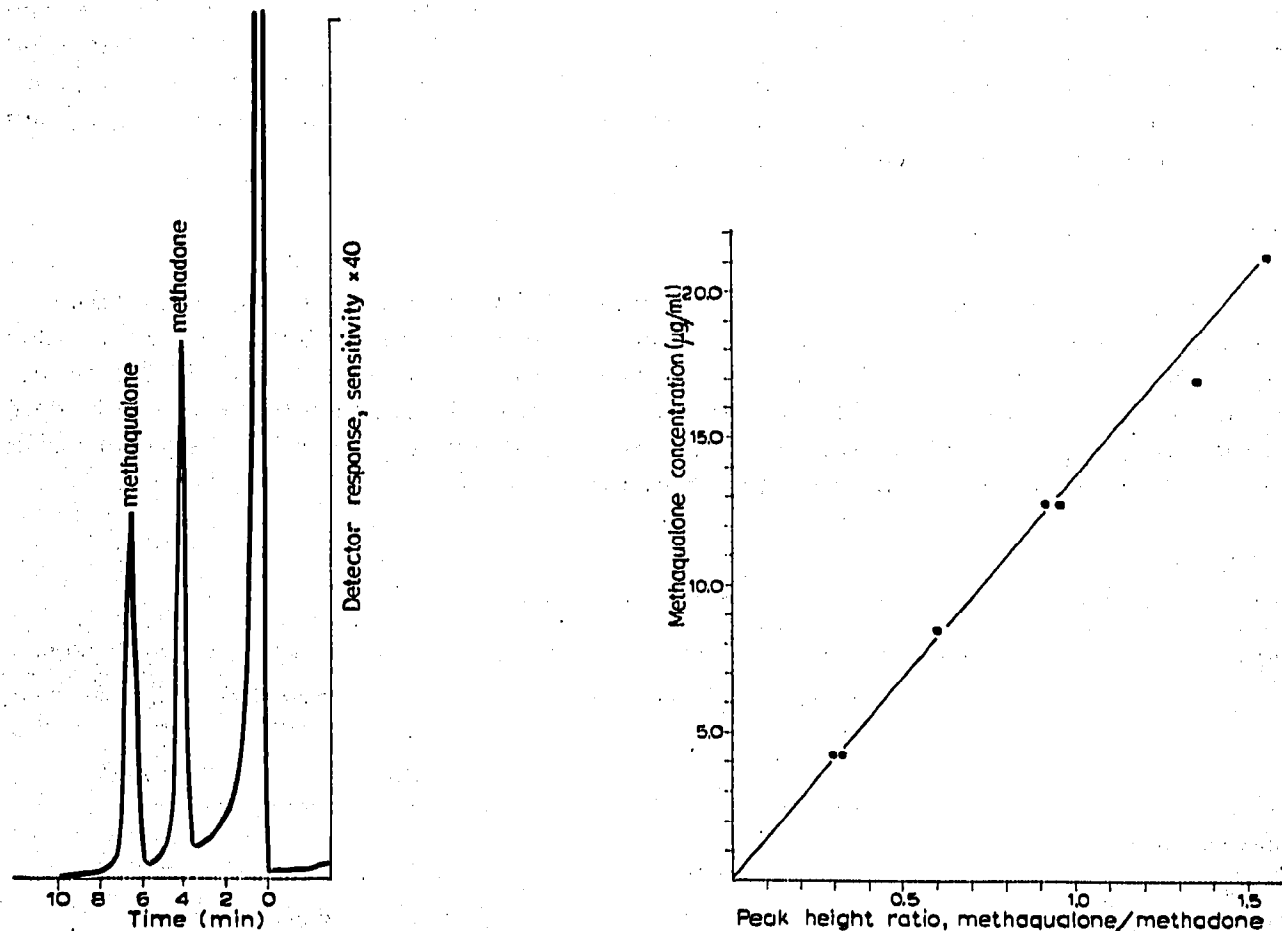


Fig. 1. The separation of methadone from methaqualone and the solvent front on an OV-17 glass column.

Fig. 2. The standard calibration graph relating the peak-height ratio of methaqualone and methadone to the number of micrograms of methaqualone in the extract.

methaqualone from plasma, after extraction into hexane, as 96.0%. The extraction procedure required long centrifugation to remove the emulsion formed, and it takes longer to concentrate hexane than diethyl ether down to microlitre volumes, although the over-all recovery from biological fluids was more efficient using hexane than diethyl ether. However, the extraction procedure with diethyl ether was rapid, any emulsion formed during the extraction was rapidly broken down and the reproducibility of the procedure better than reported by BERRY.

Application

The procedure has been used to measure methaqualone concentrations in human plasma, urine and erythrocyte after a single, oral dose of 250 mg of methaqualone powder. The blood profile of a male subject (age 26 years, weight 175 lb, packed cell volume 49%) fasted for 12 h is shown in Fig. 3. The plasma concentration reached a maximum of 2.71 $\mu\text{g}/\text{ml}$ at about 75 min after the oral dose, 75–80% of methaqualone being found in the plasma and 20–25% being taken up by the erythrocytes. The plasma:erythrocyte methaqualone ratio was not constant, but was time-dependent. Methaqualone could still be detected in plasma and urine more than 48 h later.

A more comprehensive pharmacokinetic study is at present being carried out.

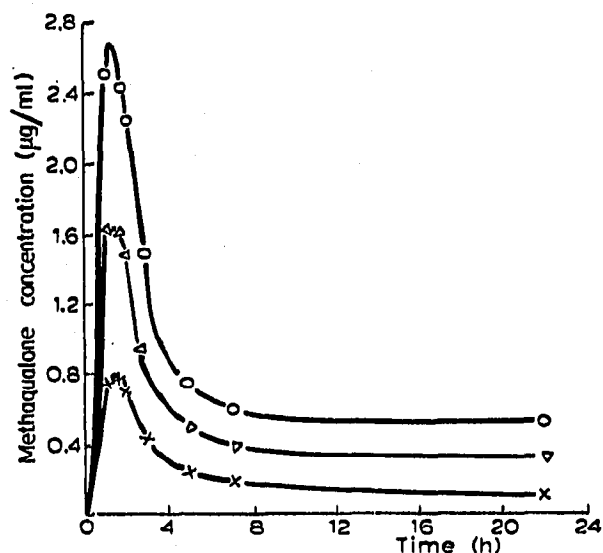


Fig. 3. Profiles of methaqualone concentrations in blood with time after a single, oral dose of 250 mg. Controlled acidic urinary pH conditions. \circ , plasma; \times , erythrocyte; ∇ , blood.

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