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GAS–LIQUID CHROMATOGRAPHY OF METHYLPENTYNOL CARBAMATE AND ITS METABOLITE 3-METHYLPENTYNE-3,4-DIOL

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SUMMARY

Procedures are described for the determination of methylpentynol carbamate in serum, either by injection into the chromatograph of diluted serum or extraction of the drug into chloroform and injection of an aliquot of the concentrated organic phase; a 4% CDMS column is used. Similar assays for measuring the metabolite 3-methylpentyne-3,4-diol in urine are reported. The methods have been used for measuring methylpentynol carbamate and its metabolite in samples from rats and dogs.

INTRODUCTION

Information on the elimination and half-life of methylpentynol carbamate (MPC) in rats and dogs is not given in the literature. Some measurements of MPC in human blood have been reported by Bartholomew *et al.*[1], but their technique gave poor recoveries. After distilling MPC from the acidified specimen into a standard solution of ammoniacal silver nitrate, they estimated the excess of silver with potassium thiocyanate, and calculated the MPC content by difference. Unfortunately, no allowance was made for the presence of any volatile metabolites containing an intact -C≡C- linkage, and it was therefore possible that this non-specific assay could give elevated results when applied to biological specimens. A similar procedure was employed by Marley and Vane[2] when MPC was administered to cats.

A sensitive and specific method for the measurement of MPC was therefore required in order to study the elimination of the drug when administered to animals. This report describes a gas chromatographic assay developed for the purpose and indicates how the excretion of a metabolite, 3-methylpentyne-3,4-diol, can be measured by using the same column.

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MATERIALS

All chemicals were of Analar grade and purchased from BDH (Poole, Great Britain), except for 2,5-dimethylhexane-2,5-diol, which was obtained from Ralph N. Emanuel (Wembley, Great Britain).

Gas–liquid chromatographic conditions

A Pye 104 chromatograph, equipped with a flame ionization detector, was used. The glass column (1.5 m × 4 mm I.D.) was silanised for 24 h with 5% dimethyldichlorosilane solution in carbon tetrachloride, and, after being emptied and dried, was packed with 4% of CDMS (Perkin-Elmer) on 100–120 mesh diatomite CLQ (J. J.'s Chromatography Ltd., King's Lynn, Norfolk, Great Britain). For MPC, the instrument settings were: injection temperature, 220°; oven temperature, 180°; detector temperature, 250°. For the MPC metabolite, the injection and oven temperatures were lowered to 180° and 140°, respectively. The attentuation was set at 1×10^{-11} A, and the gas flow-rates at all times were fixed at nitrogen 60 ml/min, hydrogen 45 ml/min and air 400 ml/min.

METHODS

Assay of MPC in serum by direct injection

Serum was diluted with an aqueous solution of 3,5-xylenol (100 mg/litre) in the proportion 4:1; as little as 40 μl of serum could be used. After mixing, 5 μl of the diluted serum was injected on to the column, the peak-height ratio was determined, and the concentration of MPC was calculated from a calibration graph constructed from the results for similar injections of standard sera (see Table I).

TABLE I

STANDARD SERA SOLUTIONS FOR PREPARATION OF CALIBRATION GRAPH FOR DIRECT-INJECTION ASSAY OF MPC

Volume of sera, μl	*Volume of aqueous MPC (1 g/l), μl*	*Volume of aqueous 3,5-xylenol (100 mg/l), μl*	*Equivalent concn. of MPC in sera, mg/l*
900	100	250	100
930	70	250	70
950	50	250	50
960	40	250	40
980	20	250	20

Assay of MPC by extraction

Serum (2 ml) was transferred, by pipette, into a glass tube (MF24/3, Quickfit and Quartz, Stone, Staffs., Great Britain), 35 ml of redistilled chloroform were added, and the mixture was shaken for 5 min. After centrifugation, the aqueous layer was rejected, the end of a glass filter-funnel was plugged with a twist of glass wool, and the chloroform was decanted through the funnel into a clean tube. Internal standard [1 ml of a solution (2 mg/litre) of 3,5-xylenol in chloroform] was added, and the solution was concentrated to *ca.* 100 μl by evaporating under a stream of nitrogen. The

evaporation was effected by transferring small portions of the organic phase to a 10-ml conical tube (BC 24/C14T, Quickfit and Quartz), care being taken to ensure that the solution never went to dryness.

An aliquot (5 μl) of the concentrated chloroform extract was injected on to the column, and the concentration was determined by calculation of the peak-height ratio of MPC to internal standard and reference to a calibration graph. A typical graph, as shown in Fig. 1, was constructed daily by injecting standard chloroform solutions prepared as detailed in Table II.

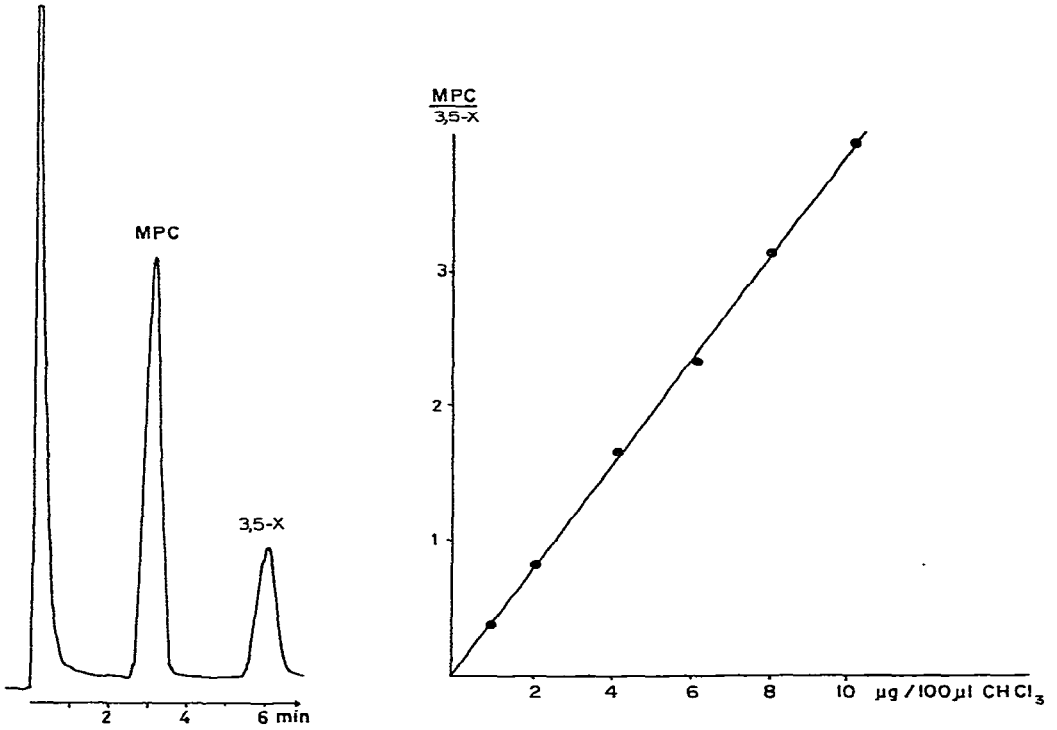

Fig. 1. Gas chromatogram and calibration graph for MPC, with 3,5-xylenol (3,5-X) as internal standard.

Assay of 3-methylpentyne-3,4-diol in urine by direct injection

Urine was diluted with a solution (100 mg/l) of 2,5-dimethylhexane-2,5-diol in the proportion 9:1. An aliquot (5 μl) was then injected on to the column (temperature 140°, see *Gas-liquid chromatographic conditions*), and the peak-height ratio of metabolite to internal standard was measured. The amount of diol was then calculated by comparison with a calibration graph prepared daily from results for standard aqueous solutions of the metabolite (10–100 mg/l) diluted with internal-standard solution in the same manner as the urine specimens.

Assay of 3-methylpentyne-3,4-diol by extraction

Urine (2–5 ml) was saturated with solid ammonium sulphate and then extracted with redistilled ethyl ether (2 × 20 ml). After centrifugation, the ether layers were

TABLE II

STANDARD SOLUTIONS IN CHLOROFORM FOR PREPARATION OF CALIBRATION GRAPH FOR ASSAY OF MPC BY EXTRACTION

Volume of MPC solution in chloroform (200 mg/l), ml	*Volume of 3,5-xylenol solution in (200 mg/l), ml*	*Volume of chloroform, ml*	*Equivalent concn. of MPC, µg/100 µl*
2.5	5	42.5	1
5	5	40	2
10	5	35	4
15	5	30	6
20	5	25	8
25	5	20	10

transferred to and combined in a clean tube in which had been placed 1 ml of a solution (10 mg/litre) of 2,5-dimethylhexane-2,5-diol in chloroform. The ether layer was then concentrated to 100 µl in a manner similar to that described previously; great care was taken to ensure that the solution did not go to dryness. Gas chromatography was performed, and the concentration of the metabolite was calculated by reference to a calibration graph established as described previously.

ANIMAL EXPERIMENTS

Rat

Twelve Sprague-Dawley rats were given an intravenous dose of 75 mg/kg of MPC after an overnight fast. Blood (100 µl) was taken at 1-h intervals from the tail vein for 5 h, and the MPC content was determined by the direct-injection technique.

Dog

A beagle was dosed intravenously with 200 mg of MPC dissolved in 10 ml of saline. Blood samples were taken at intervals from the femoral vein and assayed for MPC with use of the extraction procedure. Urine was collected for 96 h.

RESULTS AND DISCUSSION

Gas chromatography

The chromatography of MPC and its metabolite on the polar CDMS phase gave excellent symetrical peaks (see Fig. 1). At 180°, the retention times of MPC and 3,5-xylenol were 3 and 6 min, respectively, while at 140° the elution of 3-methylpentyne-3,4-diol and 2,5-dimethylhexane-2,5-diol occurred after 2.6 and 4.4 min, respectively.

The direct injection of serum and urine into the gas-chromatographic column was not ideal, and, after 40–50 injections (particularly of serum), it was preferable to repack the last 2 in. of the column and recondition it overnight. On the other hand, direct injection was very rapid and useful when numerous assays were required and the drug levels were high. It was also particularly suitable for determination of the half-

life of MPC in rat after intravenous administration; in dogs, the extraction procedure was preferred. No interfering peaks were obtained when samples of blank sera or urine were subjected to any of the methods.

Extraction and stability of specimens

It was found that, when standard solutions of MPC were made up in chloroform as described in Table II, they were stable indefinitely at room temperature. However, when evaporating chloroform solutions of MPC and internal standard (or etheral solutions of the metabolite and its internal standard), considerable losses occurred if the organic solvent was completely removed. It was essential, therefore, to ensure that solutions being concentrated were never evaporated completely to dryness.

When sera and standard sera solutions were refrigerated at −20°, no loss of MPC was observed after 2 months.

The extraction of MPC was made at physiological pH; at pH values below 4 and above 9, MPC was stable for only a few hours.

Recoveries

When known amounts of MPC were added to drug-free canine sera to give concentrations in the range 1 to 10 mg/l and these sera were carried through the extraction procedure, the recovery was 80.2 ± 2.1 %. Similarly, when 3-methylpentyne-3,4-diol was added to control urine and carried through the extraction procedure, over 95% was recovered.

Application to biological specimens

The analysis of samples of rat sera, after intravenous dosing, by the direct-injection procedure gave mean blood levels of 53 mg/l after 1 h, falling to a mean of 33 mg/l after 5 h. Table III gives the half-lives for each individual rat; the mean half-life was 4.6 h.

TABLE III

HALF-LIFE OF MPC IN TWELVE RATS AFTER INTRAVENOUS DOSING

The drug was administered at the level of 75 mg/kg.

Half-lives in individual rats, h	*Mean, h*
4.7, 4.2, 4.3, 4.2 4.5, 5.8, 4.1, 5.6 5.2, 2.0, 5.6, 5.2	4.6

Canine sera were best analysed by the extraction procedure; Table IV gives the results obtained. It was not possible to detect an α-phase, so that distribution of the drug into the tissues was presumably very rapid. The half-life of MPC was 8.3 h.

Urine measurements

Dell *et al.*[3] have indicated that the excretion of free MPC in man is low; after an oral dose of 300 mg, less than 1 % is recovered in the urine. These authors suggested

TABLE IV

SERUM LEVELS IN A BEAGLE (14 kg) GIVEN 200 mg OF MPC INTRAVENOUSLY

The half-life of the drug in this instance was 8.3 h.

Time after injection	*Concn. of MPC, µg/ml*	*Time after injection*	*Concn. of MPC, µg/ml*
3 min	13.2	3 h	9.4
6 min	11.2	4 h	8.7
10.5 min	11.3	5 h	8.7
13 min	11.3	6 h	7.4
35 min	12.6	7 h	7.2
58 min	11.2	24 h	1.7
2 h	9.7	31 h	0.9

TABLE V

EXCRETION OF MPC IN THE URINE OF VARIOUS SPECIES

Species	*Dose*	*Time specimens collected, days*	*Percentage of initial dose present as:*		
			MPC	*Diol*	*N-Glucuronide*
Man	300 mg (oral)	7	<1	13.1	76.9
Dog	200 mg (i.v.)	4	0.0	1.5	6.5
Rat	25 mg (oral)	4	6.2	0.06	0.15
Mouse	10 mg (oral)	4	9.6	10.9	10.7

that the metabolites of MPC in man are 3-methylpentyne-3,4-diol and the N-glucuronide of 4-hydroxymethylpentynol carbamate, the latter being readily hydrolysed in 0.1 *M* sodium hydroxide to the diol. A comparison of the metabolism of MPC by various species is given in Table V, which shows that only in man is most of the dose identified. Rats and dogs excrete only small quantities of the diol, and it may be that these species metabolise MPC via methylpentynol and its glucuronide.

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