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ELECTRON-CAPTURE GAS CHROMATOGRAPHY OF METHADONE AFTER OXIDATION TO BENZOPHENONE

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SUMMARY

A procedure for the determination of low levels of methadone (6-dimethylamino-4,4-diphenylheptanone-3) in serum has been developed. Methadone is extracted from serum into *n*-heptane and re-extracted into an acidic aqueous phase. Methadone is oxidized to benzophenone with barium peroxide in sulphuric acid, during which procedure an *n*-heptane phase is present into which the oxidation product is continuously extracted. The benzophenone formed is determined by means of electron-capture gas chromatography.

The recoveries are 100 ± 3% and 100 ± 4.5% at the 120 and 16 ng levels, respectively. The minimum amount that can be determined in 1 ml of serum is 4 ng.

Interferences from possible metabolites are probably minor. The main cyclic metabolite is only co-determined to a minor extent if the oxidation time is optimized. Comparison of this oxidation method with a combined gas chromatographic–mass spectrometric determination with selected ion monitoring showed identical serum levels.

INTRODUCTION

Methadone (6-dimethylamino-4,4-diphenylheptanone-3) was initially introduced as an analgesic. The use of methadone in the stabilization treatment of narcotic addicts[1] has stimulated renewed interest in the clinical pharmacology of methadone but, owing to the lack of sufficiently sensitive methods for the determination of methadone levels in plasma, the pharmacokinetics of the drug are not well known. Knowledge of plasma half lives and plasma levels of methadone in individual patients would greatly aid in the design of a proper dosage regimen for the mainte-nance treatment.

Methadone in urine samples has been determined by thin-layer chromatography with poor sensitivity[2,3]. The analysis of methadone by spectrophotometry can be performed after oxidation to benzophenone with barium peroxide[4] or cerium(IV) sulphate[5]. Although the increase in sensitivity compared with the unaltered drug is great, these methods cannot be used to establish therapeutic serum levels of metha-

done. Gas chromatographic (GC) determinations with flame ionization detection are by far the most commonly used methods for the analysis of methadone in biological specimens[6–12]. Owing to its poor chromatographic properties and low sensitivity of the detector to the drug, such methods are incapable of determining the drug in the low nanogram range.

Derivatization and electron-capture GC (GC–ECD) is known to increase the sensitivity in the determination of drugs. This paper presents a rapid and sensitive method based on the oxidation of methadone with barium peroxide in sulphuric acid to give benzophenone and the subsequent determination of this compound by GC–ECD. The selectivity of the method with regard to certain metabolites is increased by the choice of optimum oxidation conditions.

The method has been used for the determination of serum levels of methadone in patients in the maintenance treatment. A comparison of the method with a combined gas chromatographic–mass spectrometric (GC–MS) method is reported.

EXPERIMENTAL

Apparatus

A Varian 1400 gas chromatograph with an electron-capture detector (^{3}H source) was used with a glass column (150 cm × 1.8 mm) filled with 3% DC-560 + 0.3% NPGSe on Gas-Chrom P, 100–120 mesh, acid washed and silanized. The column oven temperature was 138° and the flow-rate of the carrier gas (nitrogen) was 30 ml/min. The detector temperature was 167°, corresponding to 150° at the detector foil. The injector temperature was 180°.

Chemicals

Barium peroxide, anhydrous powder, was obtained from Matheson, Coleman & Bell (East Rutherford, N.J., U.S.A.).

n-Heptane, Uvasol quality (Merck, Darmstadt, G.F.R.), was used in all extractions.

The internal standard, 4-(4-chlorophenyl)-4-phenyl-2-dimethylaminobutane, was purified as described elsewhere[13]. The methadone metabolites were received from Ely Lilly Research Labs. (Indianapolis, Ind., U.S.A.).

All other reagents were of the highest analytical purity available.

Standard solution of methadone. Methadone chloride was dissolved in 0.1 *M* orthophosphoric acid and diluted to a concentration of 32 ng/ml.

Internal standard solution. The internal standard, 4-(4-chlorophenyl)-4-phenyl-2-dimethylaminobutane, as the chloride salt, was dissolved in 0.1 *M* orthophosphoric acid and diluted to a concentration of 49 ng/ml.

Procedure for the determination of methadone in serum samples

A volume of serum corresponding to 16–120 ng of methadone (not more than 2 ml), 3.00 ml of the internal standard solution, 1 ml of 1 *M* sodium hydroxide solution and water to 5 ml are extracted with 5 ml of *n*-heptane for 30 min. After centrifugation, the organic phase is transferred into another extraction tube, 2 ml of 0.1 *M* orthophosphoric acid are added and the tube is shaken for a further 30 min. The aqueous phase is transferred into a tube containing 100 mg of barium peroxide, 1 ml

of 10.8 *M* sulphuric acid and 0.5 ml of *n*-heptane are added and the tube is attached to an air condenser and heated in a glycerol bath at 116° for 15 min. After removal of the aqueous phase, the organic phase is shaken with 2 ml of 1 *M* sodium hydroxide solution. Finally, 2–5 μl of the *n*-heptane phase are injected into the gas chromatograph.

A standard curve is constructed by treating five samples containing 0, 16, 32, 64 and 96 ng of methadone from the standard solution and 1 ml of serum according to the above procedure.

RESULTS AND DISCUSSION

Drugs that contain the diphenylmethane moiety can be oxidized with chromic acid or alkaline permanganate solution to the corresponding benzophenone[14,15]. Quantitative determinations of two drugs in serum and urine by GC–ECD are based on this principle[16,17]. On chromic acid oxidation, methadone did not form benzophenone[14]. In alkaline permanganate solution, the benzophenone formed was rapidly degraded, and the more favourable two-phase system could not be employed owing to the basic character of the drug[15].

Spectrophotometric determination of methadone after barium peroxide oxidation in a two-phase system has been performed[4] and the present method is a development of this technique.

Extraction conditions

n-Heptane is the preferred solvent for the extraction of drugs from serum as very little extraneous material is co-extracted. *n*-Heptane extracts methadone and the internal standard quantitatively at a pH exceeding 8, as can be seen in Table I. The two amines are re-extracted into 0.1 *M* orthophosphoric acid giving a pH that is more than sufficient for quantitative back-extraction into an aqueous phase. The oxidation is performed in acidic aqueous medium.

TABLE I

PARTITION COEFFICIENTS OF METHADONE AND THE INTERNAL STANDARD

$K_d = A_{org}/A_{aq}$ = partition coefficient of the amine. k_{HA} = acid dissociation constant of the amine.

Compound	*log* $(K_d \cdot k_{HA})$*	*pH for 99% extraction into n-heptane***	*pH for* $\leq 1\%$ *extraction into n-heptane***
Methadone	−5.97	≥ 8	≤ 3.9
Internal standard	−4.85	≥ 6.9	≤ 2.8

* Photometric determinations.
** Equal phase volumes.

Oxidation conditions

Benzophenone has been shown to be degraded in alkaline permanganate solution[15] and a similar degradation has also been observed in acidic barium peroxide solution[18]. Hence the benzophenone formed must be removed rapidly from the oxidation medium. If the oxidation of methadone is performed in acidic solution, a

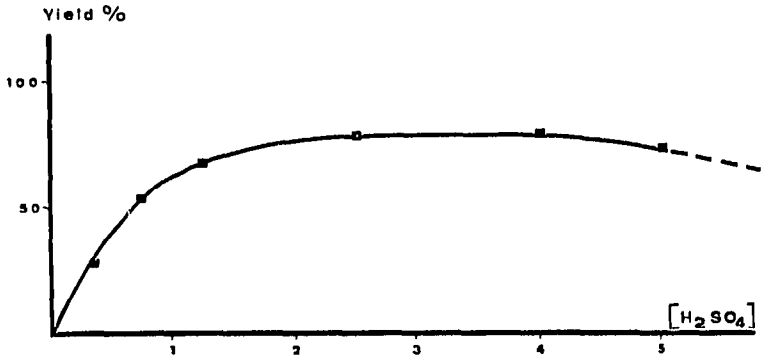


Fig. 1. Relation between concentration of sulphuric acid in the oxidation solution and oxidation yield. Concentration of methadone: 60 ng/ml. Amount of barium peroxide: 100 mg. Oxidation time: 30 min. Temperature: 116°.

two-phase system can be employed in which the ionized amine is in the aqueous phase and the neutral oxidation product is continuously extracted into the *n*-heptane phase. The following variables in the oxidation of methadone were studied.

Amount of barium peroxide. Barium peroxide has a limited solubility in aqueous solution and a study revealed a constant yield of benzophenone if the amount of barium peroxide exceeded 80 mg in 3 ml of water.

Concentration of sulphuric acid. The concentration of sulphuric acid in the oxidation solution was found to have a pronounced effect on the yield of benzophenone. The yield of benzophenone at different sulphuric acid concentrations is given in Fig. 1. The optimum yield was obtained in 2.5–4 *M* sulphuric acid; under more acidic conditions, lower yields were found.

Temperature. The oxidation was very dependent on temperature. At 60° and 90°, the yields after 30 min were 5 and 9%, respectively. In the recommended procedure, 116° is used and a constant yield of 79.1 % was obtained after 15 min, as can be seen from Fig. 2. When the temperature exceeds 100°, the aqueous phase boils,

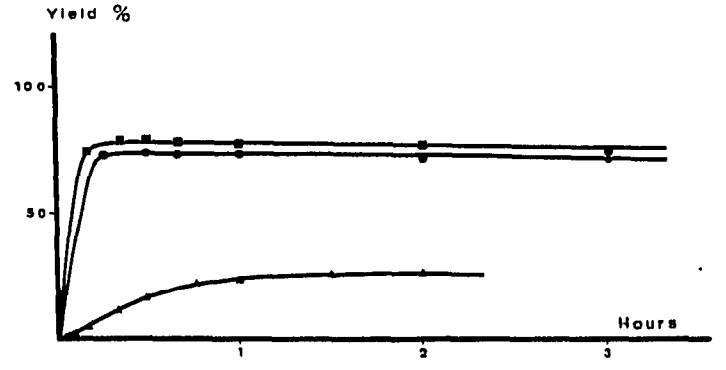


Fig. 2. Dependence of oxidation yield on time. Sample concentration: 60–100 ng/ml. Amount of barium peroxide: 100 mg Concentration of sulphuric acid: 3.6 *M*. Temperature: 116°. Compounds: ■, methadone; ●, internal standard; ▲, compound IV, Table II.

which facilitates the distribution of the benzophenone formed into the organic phase.

Volume of organic phase. Benzophenones show a very high distribution from aqueous solution into *n*-heptane, and the volume of *n*-heptane in the oxidation could be varied between 0.5 and 10 ml without influencing the yield. As a very small volume of *n*-heptane can be used, the determination of low serum levels of methadone is possible. The benzophenone blank was about 0.2 ng/ml, which is at least five times lower than in previous methods[16,17].

Oxidation time. The oxidation yields of methadone and the internal standard at different times are plotted in Fig. 2. Under the conditions used, the oxidation is very fast and a constant yield is obtained in less than 15 min. The oxidation of the internal standard needed about the same time as methadone.

With a prolonged oxidation time (3 h), a slight decrease in the yield of benzophenone, probably due to degradation, was noticed.

Yield of benzophenone and choice of internal standard

The oxidation of methadone to benzophenone is not quantitative, as can be seen from Table II. On the other hand, the yield is very reproducible (79.1 ± 1.5%) and, by suitable choice of internal standard, relative recoveries of 100% were obtained with good precision.

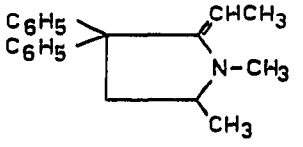
The internal standard in this study has a structure similar to that of methadone (Table II, compound V), and is oxidized to 4-chlorobenzophenone. It shows about the same distribution into *n*-heptane and the conditions for the oxidation are very similar.

Selectivity of the method

Methadone is extenstively metabolized and some of the metabolites are reported to be pharmacologically active[19]. Little information is available about the concentration of the metabolites in serum, but in urine considerable amounts of the

TABLE II

YIELD OF BENZOPHENONE FROM METHADONE AND SOME OF ITS METABOLITES ON BARIUM PEROXIDE OXIDATION

Compound	*Name*	*Oxidation yield (%)*	*Yield through the whole procedure (%)*
I	Methadone	79.1	63.2
II	6-Methylamino-4,4-diphenylheptanol-3	65.4	55.5
III	6-Dimethylamino-4,4-diphenylheptanol-3	63.2	52.6
IV	2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidene, 	9.6	~2
V	4-(4-Chlorophenyl)-4-phenyl-2-dimethylaminobutane (internal standard)	73.2*	60.7*

* 4-Chlorobenzophenone.

basic, cyclic metabolites are found[12]. Metabolites that contain the diphenylmethane moiety will be co-determined in this oxidation procedure if precautions are not taken.

Metabolites that contain hydroxyl and secondary amino groups give high oxidation yields of benzophenone, as can be seen from Table II (compounds II and III). If the metabolites are carried through the whole procedure, high yields of benzophenone are found, indicating an almost quantitative extraction of these metabolites into *n*-heptane. The difference between the yields in the two instances may be due partly to adsorption losses, as aliquots must be taken out, when the metabolites are carried through the whole procedure. These two metabolites will interfere in this oxidation method.

The main metabolite is the cyclic structure, IV, in Table II. It is very slowly oxidized, as can be seen from Fig. 2. The oxidation time in the method is 15 min, when less than 10% of the metabolite is converted into benzophenone. Therefore, this metabolite, IV, and another with similar structure will contribute to only a minor extent to the serum values found. It can be noted that none of these metabolites have been found in serum[12].

Recovery and precision

The lowest concentration of methadone that could be determined was 4 ng in 1 ml of serum.

The recovery of methadone added to blank serum was 100% and the relative standard deviation at the 120 and the 16 ng/ml levels were ± 3.0 (n = 10) and ± 4.5% (n = 6), respectively.

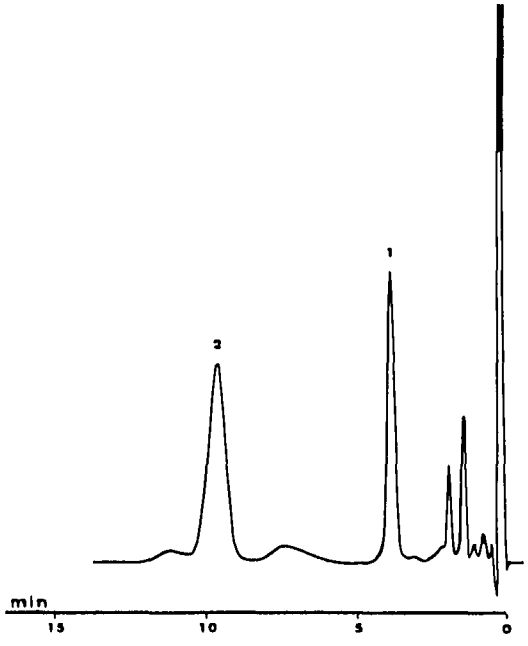


Fig. 3. Gas chromatogram of serum sample of methadone (210 ng/ml, 0.5 ml of serum) after oxidation. 1 = Benzophenone from methadone; 2 = 4-chlorobenzophenone from the internal standard.

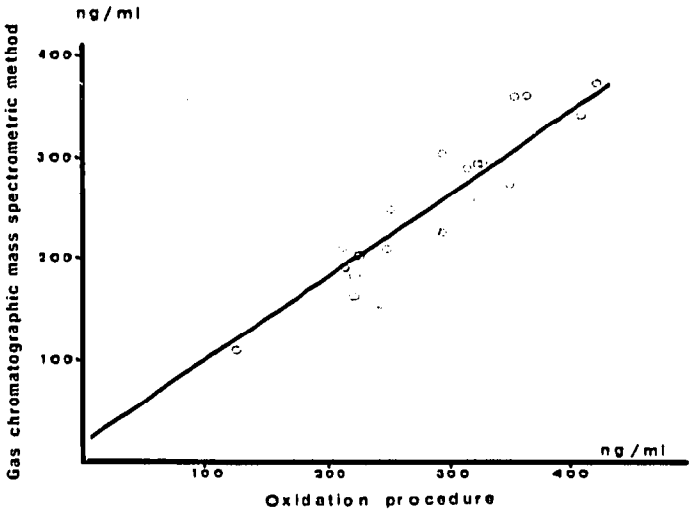


Fig. 4. Serum concentrations of methadone obtained by the GC–MS method (ordinate) and the oxidation procedure (abscissa).

Application to serum samples

This oxidation method has been used to establish levels of methadone in plasma in patients undergoing the maintenance treatment. As can be seen from Fig. 3, very clean chromatograms are obtained. Most of the extraneous material from serum is destroyed in the oxidation, and very few interferences in the determinations were found.

As this oxidation method could co-determine some minor amounts of metabolites, it was compared with a more specific method, *viz.*, GC–MS with single ion monitoring[20]. The methadone concentrations obtained from the same serum samples by the two methods are plotted in Fig. 4. The regression line had a slope of 0.85 and the intersection was at 17 ng on the GC–MS axis. The regression coefficient was 0.91. The results indicate that some benzophenone precursor contributes to a small extent to the values in the oxidation method.

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