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ELECTRON CAPTURE GAS CHROMATOGRAPHY OF METHINDIONE

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SUMMARY

A method for the determination of methindione (2-ethyl-2-methylamino-indandione-1,3) in human serum is described. The drug and a homologue as internal standard are extracted into toluene from alkaline serum and the extract is analyzed by electron capture gas chromatography. The sensitivity limit with a 2-ml sample was about 1 ng/ml. Recoveries at the 50 ng level were 100 ± 3%. Results from oral administration of a methindione solution are given.

The minimum detectable quantity (MDQ) for methindione is $3.6 \cdot 10^{-16}$ mole/sec, which implies that most drugs with the conjugated β-diketone structure are suitable for electron capture detection.

INTRODUCTION

Methindione (2-ethyl-2-methylaminoindandione-1,3) (Fig. 1) is a compound with antiepileptic properties. This compound has been determined by polarography by Vegnere *et al.*[1] in the blood serum of rats after high doses. Serum levels in man are of great interest in fully evaluating the biopharmaceutical properties of the substance.

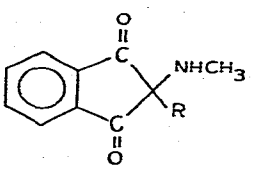

Fig. 1. Structure of methindione (R = C_2H_5) and the internal standard (R = C_3H_7).

As methindione contains two conjugated carbonyl groups, it was expected to be a good electrophore, making its detection in an electron capture detector possible. This paper presents the results of a gas chromatographic (GC) method for the determination of methindione in human serum with a sensitivity in the lower nanogram range.

MATERIALS AND METHODS

Reagents

Methindione and the internal standard (2-propyl-2-methylaminoindandione-1,3 chloride) were obtained from the Institute of Organic Synthesis, Latvian Academy of Sciences, Riga, U.S.S.R.

The extraction buffer, of ionic strength 5, was prepared by dissolving 234 g of sodium chloride in phosphate buffer (μ = 1, pH 11) and buffer was added to 1000 ml. The final pH was 9.6.

Toluene, 5 *M* hydrochloric acid and anhydrous sodium sulphate were of analytical grade.

Standard solution. Methindione chloride was dissolved in 0.1 *N* hydrochloric acid to a concentration of 500 ng/ml.

Internal standard solution. The propyl homologue of methindione chloride was dissolved in 0.1 *N* hydrochloric acid to a concentration of 500 ng/ml.

Apparatus

A Varian 1400 gas chromatograph with a tritium source electron capture detector was used.

Gas-Chrom P, 100–120 mesh, acid washed and silanized, was used as the support with three different stationary phases:

(a) 1% cyclohexane dimethanol succinate at 160°.

(b) 3% OV-17 at 160°

(c) 4% Carbowax–terephthalic acid at 190°.

The injector temperature was 170° and the detector was maintained at 180° for (a) and (b) and at 215° for (c). The nitrogen flow-rate was 30 ml/min.

Partition studies

A study of the partition properties of methindione was made with a non-partitionable reference compound as marker in the organic phase.

Partition coefficients were determined with equal phase volumes with 125 μg/ml of 4-methylbenzophenone and 280 μg/ml of methindione in the organic phase. The GC peak height ratio of the compounds in the organic phase was determined before and after equilibration with aqueous phases of different pH values. Flame ionization was used in preliminary studies. With unequal phase volumes (< 500 μl organic solvent) and with nanogram amounts of the compounds, electron capture detection was used when the organic solvent permitted that use.

Procedure

Standard samples were prepared by transferring, with a pipette, 0, 50, 100 and 200 μl of the methindione standard solution into four 12-ml centrifuge tubes fitted with standard tapered glass stoppers. To each tube were added 100 μl of the internal standard solution. To each tube were added 0.10 ml of 5 *M* hydrochloric acid and 2 ml of water and the tubes were shaken with the stoppers in place. Then 4.0 ml of the phosphate buffer were added, followed by 0.6 ml of toluene and extraction was performed for 15 min in a machine made in this labaratory.

After centrifugation at *ca.* 2000 *g* for 10 min, the aqueous phase was aspirated

and a small amount of dried sodium sulphate added to the toluene layer. About 5 μl were injected on to the column. A standard curve was constructed from peak area ratios *versus* weight ratios.

For the test samples, a volume of serum corresponding to 25–200 ng of methindione (not more than 2 ml) was diluted to 2 ml with water, 100 μl internal standard solution and 0.10 ml of 5 *M* hydrochloric acid were added and the procedure was continued as for the standard samples.

RESULTS AND DISCUSSION

Extraction properties

Preliminary experiments indicated that methindione was easily lost from solutions upon evaporation of the solvent. Concentration therefore had to be effected in the extraction steps. An ion-pair extraction step with perchlorate as the counter ion was tried, but the extractability into methylene chloride was much too low. Addition of 10% of *n*-pentanol to the organic phase gave an extraction constant of about 1 (equal phase volumes). It was therefore necessary to perform the extraction in one step into a relatively small volume of organic phase.

Partition of methindione into methylene chloride was superior to that into benzene or toluene with log $k_{d(A)} \cdot K'_{HA^+}$ values of about −3 and −4, respectively, from phosphate buffers with an ionic strength of 0.1 (for the definition of $k_{d(A)} \cdot K'_{HA^+}$, see Persson and Schill[2]). The partition of the base into toluene was found to depend considerably upon the ionic strength of the aqueous phase (Table I). These values

TABLE I

BASE PARTITION OF METHINDIONE

Aqueous phase: 1 ml of methindione (545 ng/ml) + 10 ml of buffer solution. Organic phase: 0.5 ml of toluene with *p*-chlorobenzophenone (~700 ng/ml). The peak height ratio was measured before and after equilibration of the two phases.

Ionic strength	*pH*	*Peak height ratio, Methindione:p-chlorobenzophenone*
0.1	9.9	1.42
0.5	9.4	1.40
1.0	9.15	1.39
1.5	9.0	1.45
2.9	8.6	1.57
6.1*	8.9	1.57
No extraction	—	1.59

* Saturated NaCl + phosphate buffer, pH 10.

were obtained with the very lipophilic reference compound in 0.5 ml of toluene and methindione in 11 ml of aqueous phase. An ionic strength of 2.9 or greater was sufficient to give over 99% extraction with the unequal phase volumes used. The pK_{HA^+} value of methindione has been reported[3] to be 4.63, implying that pH values above 6.6 would not influence the extraction. With the ionic strength prescribed, no change in extractability was observed above pH 6.9.

In experiments in which serum samples were spiked with methindione, it was

found that a dilution of serum with at least the same volume of water was necessary in order to recover the compound quantitatively.

Gas chromatography

The amine character of methindione is rather weak, which was apparent in the GC step. On the relatively non-polar phase OV-17, sharp symmetrical peaks were obtained even with nanogram amounts. Cyclohexane dimethanol succinate and Carbowax–terephthalic acid could also be used in those instances where some interfering peaks appeared on OV-17 in the vicinity of methindione and the internal standard. The successful use of the latter phase is of interest (Fig. 2).

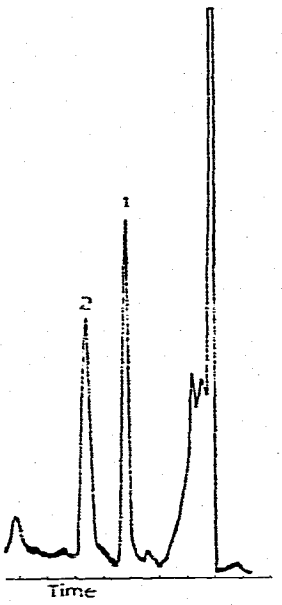

Fig. 2. Gas chromatogram of a toluene extract from a 0.25 ml serum sample containing 162 ng ml of methindione. About 350 pg were injected. 1 = Methindione; 2 = internal standard.

Electron capture properties

Methindione showed a good response in the electron capture detector used. A minimum detectable quantity (MDQ) value of $3.6 \cdot 10^{-16}$ mole/sec was observed at the ordinary detector temperature of 180°, which is comparable with that of many well known derivatives such as the heptafluorobutyryl derivatives as described by Walle and Ehrsson[4].

Preliminary studies of the response at 150° and 215° showed that the peak height was insensitive to these temperature changes. The type of detection mechanism[5] cannot be elucidated from these few observations. A behaviour similar to that of the phthalates and some benzophenones would be expected but in the detector used they showed a decrease in response even at the temperatures used in this work[6].

Stability of methindione in samples

The risk of hydrolysis of methindione in the samples before analysis must be

considered as the dione ring will easily open at pH values above 4 (ref. 7). When samples containing 175 ng/ml of methindione were stored at room temperature, the amount present decreased to 60 ng/ml in 13 days. This degradation could be reduced considerably by the addition of 0.1 ml of concentrated phosphoric acid per 2 ml of sample after the separation of the erythrocytes. This acid did not precipitate the serum proteins. If, on the other hand, the samples were stored deep frozen and thawed five times during 3 weeks, no decrease in content could be demonstrated. From a practical point of view, the latter procedure was preferred.

Internal standard

The use of a homologue to methindione as the internal standard was of utmost importance for successful analysis, especially with the use of a small volume of organic phase in a single extraction step. This concept has been used successfully in several bioanalytical applications, with and without previous derivative formation.

Application to serum samples

Upon addition of methindione to serum samples, recoveries of 100 ± 3% were obtained at the 50 ng/ml level. When samples from an actual experiment in man were analyzed, it was observed that dilution of the samples with at least the same volume of water was not sufficient to release all of the drug present. Consistent and reproducible values were obtained when the samples were acidified before alkalination and extraction. The increase in concentration varied from two to five times. The reason for this increase is not clear, but some kind of protein binding might be involved.

An example of the time course for the serum concentration of methindione after oral administration of a solution is shown in Fig. 3. The serum half-life is fairly short.

A number of other drugs are conjugated β-diketones and it should then, in

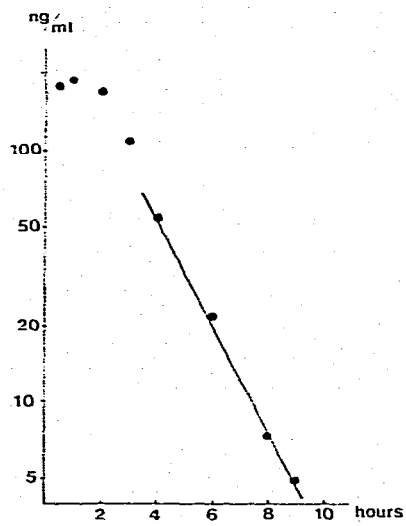

Fig. 3. Time course for the concentration of methindione in serum after an oral dose of 250 mg.

principle, be possible to determine them by electron capture detection in the lower nanogram range.

Experiments with phenylindandione showed that, after methylation of the acid function, the GC properties were good and the sensitivity comparable with that of methindione. The methylation was preferably carried out by the extractive alkylation technique[8].

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