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# DETERMINATION OF 8-METHOXYPSORALEN IN PLASMA BY ELECTRON CAPTURE GAS CHROMATOGRAPHY

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## SUMMARY

A method is given for the determination of 8-methoxypsoralen in human plasma at the low ng/ml level using gas chromatography with electron capture detection. 8-Methoxypsoralen was extracted from plasma with methylene chloride at pH 7.0. After addition of the internal standard, 8-butoxypsoralen, the psoralens were hydrolysed in sodium hydroxide and the aqueous phase was purified by extraction with methylene chloride and toluene. The aqueous phase was acidified and the re-lactonized psoralens were extracted with toluene and analysed.

Some determinations of plasma levels of 8-methoxypsoralen after oral administration are presented.

## INTRODUCTION

Psoralens have been used in the therapy of vitiligo for more than two decades. Recently increased interest has been shown in their use in the treatment of psoriasis. 8-Methoxypsoralen (8-MOP, Fig. 1) has been used both in local[1] and peroral[2,3]

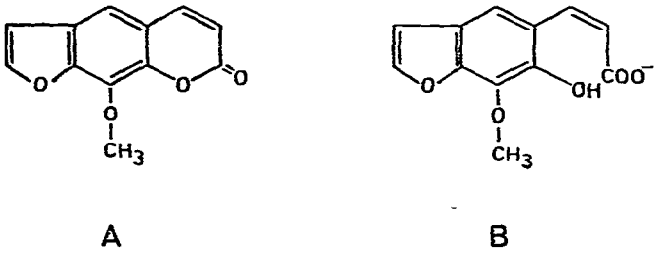


Fig. 1. A: 8-methoxypsoralen (8-MOP); B: *cis*-3-(6-hydroxy-7-methoxy-5-benzofuranyl)acrylic acid (8-MOP-acid).

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therapy, combined with exposure of the patients to long-wavelength UV light. Little information is available concerning the pharmacokinetics of 8-MOP owing to the lack of analytical techniques with sufficient sensitivity and selectivity. This paper gives a method for the determination of 8-MOP in human plasma at the low ng/ml level.

## EXPERIMENTAL

### *Apparatus*

The gas chromatographic (GC) analysis was performed using a Varian 1400 instrument equipped with an electron capture detector (ECD) of $^{63}$Ni-type. The following temperatures were used: injector, 250°; column, 195°; detector, 200°.

A silanized glass column (90 cm × 2 mm I.D.) was packed with 3% OV-225 on Gas Chrom Q, 100/120 mesh. Nitrogen was used as carrier gas with a flow-rate of 20 ml/min. The column was conditioned for 24 h at 275°.

Photometry was performed with a Zeiss PMQ III Spectralphotometer equipped with thermostated cells (10 mm).

The pH determinations were performed with an Orion Research Model 701/ digital pH meter equipped with an Ingold combined electrode, Type 401.

The radioactivity was measured with a Packard Tri Carb instrument, Model 3320.

### *Chemicals*

All solvents were of analytical grade. Aqueous and organic phases were equilibrated before use in partition experiments. 8-MOP was supplied by AB Draco, Lund, Sweden.

8-Butoxypsoralen (8-BOP) was synthesized as follows: 8-hydroxypsoralen (25 mg, prepared from 8-MOP by demethylation[4]) in 25 ml of methylene chloride was mixed with 5 ml of butyl iodide and 10 ml of carbonate buffer (pH 10.5) containing 1 *M* tetrabutylammonium hydrogen sulphate (neutralized with an equivalent amount of sodium hydroxide). The mixture was shaken for 30 min at room temperature. The organic phase was separated and extracted three times with 10 ml of buffer (pH 10.5) and twice with water and dried with $Na_2SO_4$. The organic solvent was evaporated and the residue recrystallized from methanol–water. The structure was confirmed by mass spectrometry.

Tritium-labelled 8-MOP was synthesized from 8-hydroxypsoralen and [$^3H_3$]-methyl iodide[4] and purified by thin-layer chromatography (TLC) (Silica gel 60, chloroform, $R_F = 0.5$) immediately before use. The radiochemical purity was >99%, as checked by TLC with radiochromatogram scanning.

8-Methoxypsoralen tablets (Neomeladinine, 15 mg) were obtained from the Memphis Chemical Co. (Cairo, Egypt). The doses given were 30 mg to patients weighing 35–55 kg, 45 mg to patients weighing 55–75 kg and 60 mg to patients weighing more than 75 kg.

### *Determination of partition coefficients*

The organic phase containing 8-MOP was equilibrated with phosphate buffer pH 7.0 ($\mu = 0.1$) for 30 min at 25° (equal phase volumes). The concentration of

8-MOP was determined photometrically: in the organic phase by direct measurements and in the aqueous phase after re-extraction into methylene chloride.

*Plasma extractions*

The extraction from plasma was studied using tritium-labelled 8-MOP (426 mCi/mmole). The radioactivity in aqueous and organic phases was determined by liquid scintillation counting after addition of Instagel®.

The plasma extraction of 8-butoxypsoralen was studied by GC–ECD using 8-MOP as internal standard.

*Determination of apparent first-order rate constants for hydrolysis and lactonization*

The constants were determined by photometry (*cf.* ref. 5).

The lactonization was studied at 25.0° by mixing 0.200 ml of hydrolysed 8-MOP in 0.01 *M* NaOH with 3.00 ml of the appropriate buffer ($\mu = 0.1$, thermally equilibrated) in the thermostated cells of the spectrophotometer and following the change in absorbance at 300 nm. The absorbance at $t_\infty$ was obtained after the addition of 0.100 ml of 12 *M* HCl.

The hydrolysis was studied as described above by mixing 3.00 ml of an aqueous solution of 8-MOP with 0.300 ml of the appropriate buffer ($\mu = 1$). Hydrolysed 8-MOP in buffer (pH 7.0) and 0.01 *M* NaOH exposed to light for 2 h at 300 nm in the photometer gave, after acidification, the same spectra as a freshly prepared solution of 8-MOP, thus indicating no photochemical degradation.

*General procedure for the determination of 8-MOP in plasma*

A 2.00 ml plasma sample was mixed with 0.5 ml of phosphate buffer (pH 7.0, $\mu = 1$) and 5.00 ml of methylene chloride and extracted for 15 min. Then 3.00 ml of the organic phase was mixed with 0.200 ml of 8-BOP (internal standard) 125 ng/ml in methanol and evaporated to dryness under nitrogen, after which 0.025 ml of methanol and 0.200 ml of 0.1 *M* NaOH were added and the solution was left for 10 min at room temperature. The aqueous phase was extracted once with 1 ml of methylene chloride (discarded) and twice with 1 ml of toluene (discarded). The aqueous phase was mixed with 0.050 ml of toluene and 0.025 ml of 12 *M* HCl and extracted for 3 min. A 2 $\mu$l sample of the organic phase was injected into the chromatograph.

All quantitations were based on peak height measurements.

Blood samples (10 ml) were obtained by venous puncture. After centrifugation the plasma was immediately frozen (−20°). Handling of plasma samples at room temperature was kept to a minimum.

## RESULTS AND DISCUSSION

The method consists of extraction of plasma with methylene chloride and separation of 8-MOP from endogenous compounds by extraction, followed by GC determination using an ECD. The high sensitivity of the ECD can be fully utilized only after separation of 8-MOP from co-extracted endogenous material prior to the GC step. A selective extraction was obtained because psoralens hydrolyse in alkaline and re-lactonize in acidic solutions.

TABLE I

PARTITION COEFFICIENTS FOR 8-METHOXYPSORALEN

Aqueous phase: phosphate buffer, pH 7.0, $\mu = 0.1$. Temperature: 25°.

| *Organic phase* | *log* $k_d$ |
|---|---|
| Methylene chloride | >3 |
| Benzene | 2.7 |
| Toluene | 2.5 |
| Diethyl ether | 1.8 |
| Hexane | 0.60 |

*Extraction*

The partition coefficients for 8-MOP are given in Table I. Quantitative extraction (>99%) is obtained with methylene chloride, benzene or toluene using equal phase volumes. The internal standard, 8-BOP, should give higher partition coefficients owing to its higher carbon content (*cf.* ref. 6). Methylene chloride was preferred as solvent owing to its good extracting properties and high volatility, facilitating subsequent evaporation.

Extraction studies from plasma revealed, however, a lower recovery compared to aqueous solutions (Table II). This was particularly notable for 8-BOP, which was extracted to *ca.* 70% only (equal phase volumes). The lower extraction of 8-BOP

TABLE II

EXTRACTION OF PSORALENS FROM PLASMA

Organic phase: methylene chloride. Aqueous phase: plasma pH 7.0 (2.00 ml plasma + 0.5 ml phosphate buffer, pH 7.0, $\mu = 1$). Extraction time: 15 min.

| *Ratio* $\frac{org.\ phase}{aq.\ phase}$ | *Conc. (ng/ml)* | *Yield (%) (n = 6)* |
|---|---|---|
| *8-Methoxypsoralen* | | |
| 1:1 | 100 | 91.7 ± 0.2* |
| 2.0:1 | 100 | 93.4 ± 0.2* |
| 2.0:1 | 10 | 91.4 ± 0.1* |
| *8-Butoxypsoralen* | | |
| 1:1 | 100 | 72 ± 5** |

* Determined by liquid scintillation counting.
** Determined by GC–ECD.

TABLE III

APPARENT FIRST-ORDER RATE CONSTANTS FOR HYDROLYSIS OF 8-METHOXYPSORALEN (8-MOP) AND 8-BUTOXYPSORALEN (8-BOP)

Temperature: 25°. Each value is a mean of two determinations.

| *pH* | $-$ *log* [$k$ ($sec^{-1}$)] | |
|---|---|---|
| | *8-MOP* | *8-BOP* |
| 10.25 | 3.96 | — |
| 10.94 | 3.27 | — |
| 12 (0.01 *M* NaOH) | 2.34 | 2.41 |

TABLE IV

APPARENT FIRST-ORDER RATE CONSTANTS FOR LACTONIZATION OF 8-MOP-ACID AND 8-BOP-ACID

Temperature: 25°. Each value is a mean of two determinations.

| *pH* | $-\log [k\ (sec^{-1})]$ | |
|---|---|---|
| | *8-MOP-acid* | *8-BOP-acid* |
| 1.95 | 1.93 | 1.99 |
| 2.98 | 2.74 | — |
| 4.34 | 3.71 | — |
| 7.07 | 4.51 | — |

compared to 8-MOP may be due to a higher degree of plasma protein binding (*cf.* ref. 7). Because of its different plasma extraction properties 8-BOP was added after the initial extraction. A ratio of organic phase to plasma of 2.0 gave an extraction of 8-MOP of 93.4% at 100 ng/ml and 91.4% at 10 ng/ml. The degree of extraction was not increased by varying the extraction time from 15 min to 60 min.

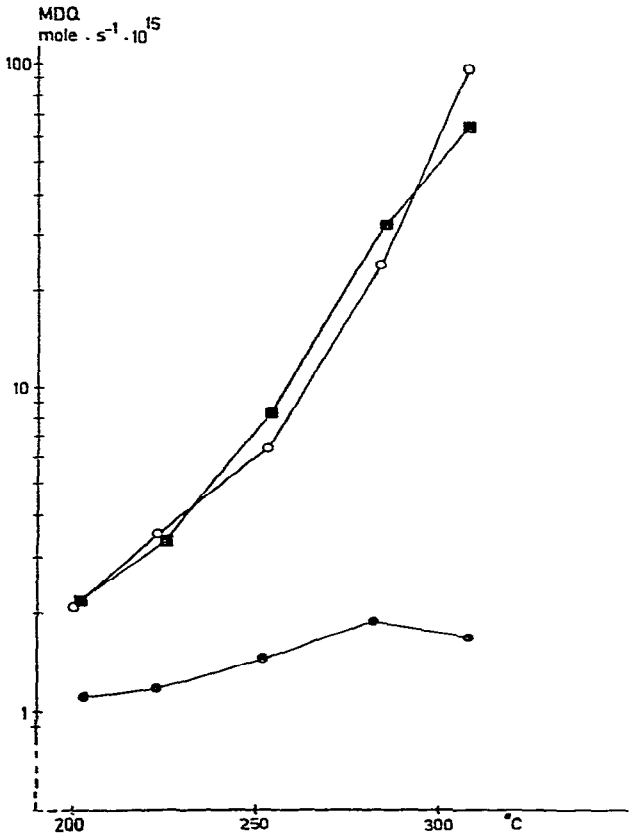


Fig. 2. Electron capture detector response for psoralens as a function of detector temperature. ○: 8-Butoxypsoralen; ■: 8-methoxypsoralen; ●: 5-methoxypsoralen.

*Hydrolysis and lactonization*

8-MOP is hydrolysed in alkaline solutions to 8-MOP-acid (Fig. 1, *cf.* ref. 8). The apparent first-order rate constants for hydrolysis at three different pH values are given in Table III. At pH 7.0 8-MOP was stable for at least 24 h but in 0.1 *M* NaOH hydrolysis was complete in <3 min.

The rate of lactonization at various pH values is given in Table IV; it was complete within 1 min in 0.1 *M* HCl.

The rates of lactonization and hydrolysis of the internal standard, 8-BOP, differed only slightly from those of 8-MOP (Tables III and IV).

*Electron capture detector response*

The minimum detectable quantity (MDQ, signal-to-noise ratio 3:1) for 8-MOP, 8-BOP and 5-methoxypsoralen (5-MOP) are given in Fig. 2. The responses for 8-MOP and 8-BOP are strongly affected by the detector temperature: a decrease of the detector temperature from 300° to 200° enhanced the response *ca.* 30-fold. The response of 5-MOP is only slightly temperature-dependent.

The MDQ for 8-MOP at 200° corresponds to an injected amount of 6 pg ($N = 3600$, $t_R = 3$ min).

*Precision and selectivity*

A chromatogram from a plasma sample obtained from a patient 6 h after an oral dose of 30 mg 8-MOP and containing 45 ng/ml of 8-MOP is given in Fig. 3. No peaks interfering with the analysis of 8-MOP were observed when analysing

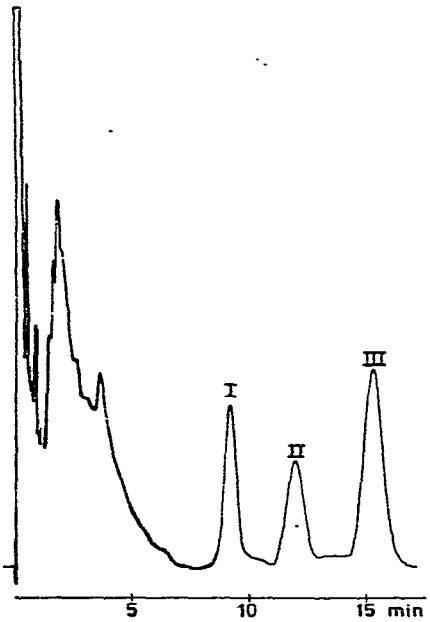


Fig. 3. Chromatogram from patient plasma containing 8-methoxypsoralen at a concentration of 45 ng/ml. For chromatographic conditions see Experimental section. I: 8-Methoxypsoralen; II: unknown compound; III: 8-butoxypsoralen.

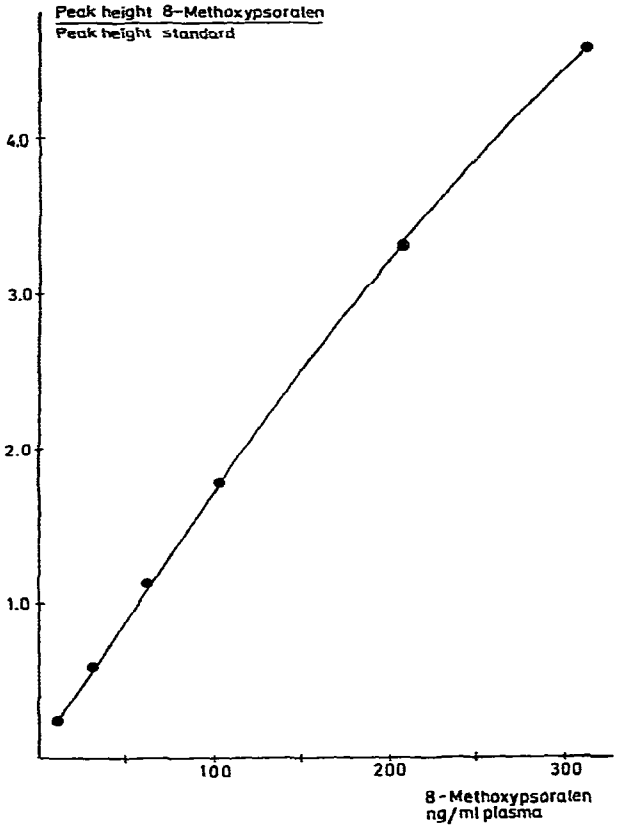


Fig. 4. Standard curve for the determination of 8-methoxypsoralen in plasma.

blank plasma. A standard curve for the determination of 8-MOP in plasma is given in Fig. 4. The relative standard deviation was 2.1% at 100 ng/ml and 4.2% at 10 ng/ml ($n = 5$).

8-Hydroxypsoralen, a likely metabolite of 8-MOP, did not interfere as it has a longer retention time than 8-BOP.

It is reasonable to assume that 8-MOP can be metabolized to some extent by esterases to 8-MOP-acid, which can then spontaneously re-lactonize to 8-MOP. The interference of 8-MOP-acid in the determination of 8-MOP will depend on their relative concentrations and the way the plasma samples are handled (*e.g.* storage time and temperature, pH used for the extraction). Plasma concentrations of 8-MOP-acid were determined in three patients, 1, 3 and 6 h after an oral dose of 30 mg of 8-MOP, using a method based on ion pair extraction and GC–ECD[9], but in all cases were below the detection limit of the technique (<10 ng/ml plasma).

*Plasma concentrations of 8-MOP*

The plasma levels of 8-MOP in five patients receiving 8-MOP orally are given in Fig. 5 and show considerable variation in the peak plasma levels (20–700 ng/ml). Further pharmacokinetic studies are in progress.

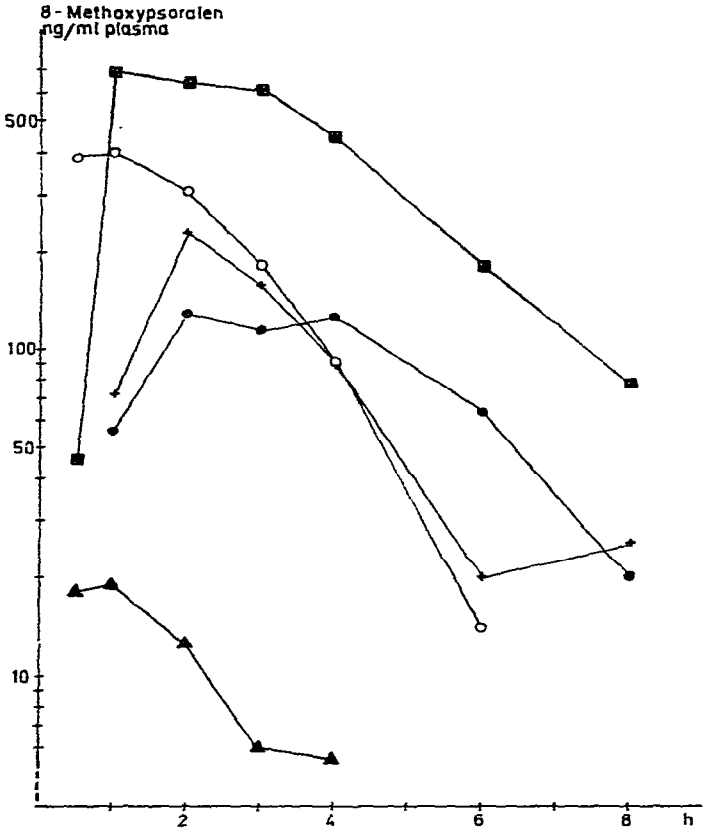


Fig. 5. Plasma levels of 8-methoxypsoralen.

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