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Letter to the Editor

Determination of psoralen in serum by reversed-phase high-performance liquid chromatography

Sir,

Psoralen, 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP) and 4,5′,8-trimethylpsoralen (TMP) are furocoumarins, also called psoralens. These compounds are used in dermatology for the treatment of various skin disorders, especially psoriasis and vitiligo [1,2]. The treatment consists usually of oral intake of a psoralen followed by irradiation with longwave ultraviolet light (UV-A, 320–400 nm) and is commonly called PUVA (Psoralen UV-A). 8-MOP in particular has become an accepted treatment modality for psoriasis during the last ten years and is the most widely used of the psoralens. The therapeutic effectiveness of psoralen in the treatment of vitiligo has recently been described [3].

Data about the analysis in biological fluids and about pharmacokinetics of 8-MOP, 5-MOP and TMP are available in the literature [2].

To our knowledge no such data are available about psoralen. We therefore developed a high-performance liquid chromatographic (HPLC) method for the determination of psoralen, based on a method for the determination of 8-MOP, described earlier in literature [4]. Because of the volatility of psoralen some special precautions had to be taken for the extraction procedure.

EXPERIMENTAL

Chemicals

All chemicals used were analytical grade (Merck, Darmstadt, F.R.G.), unless otherwise specified. Psoralen was obtained from Promedica (Levallois-Perret, France). 5-MOP was obtained from Memphis Chemicals (Cairo, Egypt).

Apparatus

The liquid chromatograph consisted of a Model 510 pump, a Model U6K injector, a Model 481 UV detector and an Omniscribe recorder (Waters Millipore, Etten-Leur, The Netherlands). Separations were carried out on a 15 cm × 4.6 mm I.D. stainless-steel column containing 5-μm silica coated with octadecylsilane (Supelcosil, LC18, Supelco, Middelburg, The Netherlands).

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Standard solutions

A standard solution of 10.0 mg/l psoralen in methanol was used. The internal standard solution was a solution of 20.0 mg/l 5-MOP in methanol. The linearity and the precision of the assay were determined by adding respectively 0, 5, 10, 15 and 20 μl of the standard psoralen solution to 1.0 ml of drug-free serum. These samples were treated as described under *Assay procedure*. Ratios of the peak heights of psoralen and the internal standard were measured, and a calibration line was constructed.

Glassware

Glass test-tubes (10 ml) and glass conical-shaped distilling flasks (25 ml) were used. The standard and the internal standard solutions were dispensed by an analytical syringe (S.G.E., Middelburg, The Netherlands).

Assay procedure

Method 1. All chromatography was done at ambient temperature. The mobile phase consisted of methanol–water (60:40) and was degassed before use by filtration through a 0.22-μm membrane filter by suction. The flow-rate was 1.0 ml/min. Serum (1.0 ml) and internal standard solution (10 μl) are mixed in glass test-tubes, and 5 ml of heptane–dichloromethane (4:1) are added and shaken for 2 min. The mixture is centrifuged for 5 min at 2700 *g*, and ca. 4 ml of the upper organic layer are transferred to a clean conical-shaped distilling flask. Distilled water (100 μl) is added, and the organic solution is evaporated under reduced air pressure on a 50°C water-bath with a Rotavapor (Pleuger, Amstelveen, The Netherlands).

The distillation process is stopped just after evaporation of the organic solution, and 100 μl of methanol are added to the aqueous residue. The mixture is mixed on a vortex mixer for 30 s, and 15 μl are injected into the high-performance liquid chromatograph.

Method 2. The chromatographic conditions are the same as for method 1 except that the mobile phase now consisted of methanol–water (55:45). The same extraction procedure is used, but ca. 4 ml of the organic solution are transferred to a glass test-tube and 50 μl of polyethyleneglycol (PEG 400) are added. The organic solution is evaporated on a 50°C water-bath under a nitrogen stream. Then 200 μl of mobile phase are added to the PEG 400 residue, and the solution is mixed on a vortex mixer for 30 s. The HPLC injection volume is 20 μl.

Recovery studies

Recoveries of psoralen from serum at 100 and 200 μg/l were determined by adding the internal standard solution (10 μl) after extraction with 5.0 ml heptane–dichloromethane (4:1) to 4.0 ml of the extract solution. The peak-height ratios were calculated (R_1). These figures were compared with peak-height ratios (R_2) obtained by injecting a solution of, respectively, 100 ng of psoralen and 200 ng of 5-MOP and 200 ng of psoralen and 200 ng of 5-MOP per 200 μl mobile phase. The recovery is $R_1/R_2 \times 1.25 \times 100\%$. In a similar way recoveries of 5-MOP from serum were determined at 200 μg/l. In this case 100 ng of psoralen were added after extraction.

TABLE I

PEAK-HEIGHT RATIOS AND RECOVERIES AT DIFFERENT CONCENTRATIONS

Values in parentheses are coefficients of variation.

Concentration (μg/l)	Peak-height ratio (mean, $n=4$)		Recovery (mean ± S.D., $n=3$) (%)	
	Method 1	Method 2	Method 1	Method 2
Psoralen				
50	0.59 (7%)	0.73 (4%)		
100	1.15 (5%)	1.49 (5%)	103 ± 4	101 ± 1
150	1.81 (6%)	2.24 (3%)		
200	2.42 (2%)	3.02 (4%)	99 ± 5	104 ± 4
5-MOP				
200			101 ± 7	96 ± 5

TABLE II

RECOVERY OF PSORALEN AFTER DIFFERENT EVAPORATION TIMES

Time (min)	Ratio (mean ± S.D., $n=3$)	Recovery (mean ± S.D., $n=3$) (%)
1	1.26 ± 0.01	95 ± 1
2	0.98 ± 0.03	74 ± 3
3	0.63 ± 0.20	48 ± 20

RESULTS AND DISCUSSION

For method 1 the capacity factors for psoralen and 5-MOP were 2.2 and 3.2, respectively, and the total analysis time for one run was ca. 6 min. For method 2 the capacity factors were 3.0 (psoralen) and 5.4 (5-MOP), and the total analysis time was 9 min.

Table I gives the mean peak-height ratios at different concentrations and the recoveries from the spiked serum samples found with both methods. A linear relationship was observed between the ratio of the peak heights and the psoralen serum concentrations in the range 50–200 μg/l. The standard line for method 1 is described by the equation $y=0.012x-0.016$ (y=ratio, x=psoralen concentration, μg/l) and for method 2 by the equation $y=0.015x-0.014$.

The coefficient of correlation was 0.9975 (method 1) and 0.9984 (method 2). The detection limit, defined as thrice the noise level at 0.01 a.u.f.s., is 6 μg/l.

At first we evaporated the organic extract to dryness on a 50°C water-bath under a nitrogen stream without precautions. However, under these circumstances psoralen loss and large variation of the results were observed. Table II lists the ratios after different evaporation times. After longer evaporation times the ratios become lower, indicating the disappearance of psoralen. This phenomenon can be explained by the evaporation of psoralen. For the determination of the serum concentrations of volatile drugs special precautions have to be taken [5],

such as careful evaporation or addition of a non-volatile solvent to the extraction solvent in order to block evaporation of the drug. Method 1 is a combination of careful evaporation and partial blocking of evaporation by the addition of water. After addition of water, evaporation under reduced air pressure and stopping of the distillation process at the moment that all organic solvent has been evaporated ensured that no psoralen loss was measured. With method 2 careful evaporation is not necessary because the added PEG 400 effectively prevents evaporation. However, the presence of PEG 400 disturbs HPLC separation, therefore it was necessary to change the mobile phase, leading to a longer analysis time.

The determination methods described are easy to perform, rapid and sensitive, and seem suitable for the routine monitoring of psoralen concentrations and pharmacokinetic studies.

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