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Short Communication

Determination of 8-methoxypsoralen in plasma by gas chromatography–mass spectrometry using selected-ion monitoring

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ABSTRACT

A sensitive and accurate assay was developed for the measurement of 8-methoxypsoralen in plasma using electron-impact positive-ion mass fragmentography. 4,5,8-Trimethylpsoralen was used as an internal standard. Sample preparation consisted of a two-step liquid phase extraction using acetonitrile and methylene chloride. The calibration curve showed a linear relationship between the peak areas of 8-methoxypsoralen and 4,5,8-trimethylpsoralen over a wide range of 8-methoxypsoralen concentrations (1–500 ng/ml). Within- and between-run precisions, measured at five different drug concentrations, varied from 0.82 to 1.41% and from 0.82 to 1.86%, respectively.

INTRODUCTION

The combination of 8-methoxypsoralen (8-MOP) and ultraviolet-A light (UVA) represents a common therapeutic approach to the management of hyperproliferative diseases of the skin, such as psoriasis [1]. More recently, 8-MOP plus UVA light has been used successfully in an extracorporel system (named photopheresis) for the treatment of cutaneous T cell lymphoma (CTCL) and several autoimmune diseases [2,3].

The efficacy of all the photochemotherapies involving 8-MOP and UVA is demonstrated to be strictly related to the amount of light delivered and to the drug level in the target tissues [4]. The plasma concentration of the drug has to be monitored, during the therapy, because of the important intra- and interindividual differences in the absorption of psoralen and the different pharmacokinetics of the 8-MOP formulations used.

Plasma psoralen levels are routinely measured by high-performance liquid chromatography (HPLC) after a preliminary extraction with organic solvents or solid-phase cartridges [5–10]. The liquid phase extractions are usually time-consuming and involve several steps; on the other hand, the solid-phase cartridges allow a rapid extraction, but the addition of [^{3}H]-8-MOP as internal standard is needed to calculate the recovery of 8-MOP in the sample [9]. Another procedure involving solid-phase extraction of the specimen has been recently described. It is simple and does not require radiolabeled 8-MOP to verify the drug recovery, but the method shows a poor precision with a coefficient of variation (C.V.) of 10% at an 8-MOP concentration of 50 μg/l [10].

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There are few reports on the application of gas chromatography–mass spectrometry (GC–MS) in the analysis of psoralens in biological fluids [11,12]; in addition no GC–MS methods have been used until now to measure the 8-MOP level in the blood of patients treated with 8-MOP and UVA (PUVA).

We describe a rapid and sensitive GC–MS procedure for the 8-MOP determination in the blood after a simplified liquid phase extraction; 4,5,8-trimethylpsoralen (TMP) is used as an internal standard.

EXPERIMENTAL

Patients

Plasma 8-MOP levels of patients undergoing photopheresis were determined after ingestion of 0.6 mg/kg of 8-MOP (Oxsoralen, Gerot, Vienna, Austria). A 2-ml blood sample was drawn in a heparinized syringe at 0, 0.5, 1, 2, 4, 6, 9, 12 h after administration of the drug. The plasma was separated and stored at −20°C until use.

Chemicals

Acetonitrile (LiChrosolv grade) and methylene chloride were from Merck (Darmstadt, Germany). TMP and 8-MOP were from Sigma (St. Louis, MO, USA).

GC–MS apparatus

The GC–MS apparatus used was a Kratos Model MS25RF mass spectrometer (Manchester, UK), equipped with a Carlo Erba MFC500 gas chromatograph (Milan, Italy) and a DS90 data analysis system (Data General, Westboro, MA, USA).

Sample preparation

Plasma (1 ml), after addition of an internal standard (TMP, 50 ng in 50 μl of ethanol), was mixed with an equal volume of acetonitrile. The mixture was shaken strongly for 30 s and then left for 10 min at −20°C. After centrifugation at 1200 *g* for 10 min the supernatant was removed and freeze-dried.

The residue was dissolved in 1 ml of water and extracted twice with 2 ml of methylene chloride. The two organic phases, obtained by shaking the mixture for 1 min and then centrifuging for 10 min at 1200 *g*, were combined and freeze-dried. The residue was reconstituted in 50 μl of methylene chloride, and 1.5 μl were analysed by GC–MS (split ratio 10:1).

Standard solutions and calibration curve

A stock solution of 8-MOP (1 mg/ml) and a series of dilutions (0.02, 0.2, 2, 20 μg/ml) in absolute ethanol were prepared. A calibration curve with a concentration range of 1–500 ng/ml of 8-MOP was obtained by adding 50 μl of the different standard solutions to 1 ml of plasma. An aliquot of TMP (50 ng in 50 μl of absolute ethanol) was added to the samples as internal standard.

GC–MS procedure

A capillary HP ULTRA2 (crosslinked 5% phenylmethylsilicone) column, 25 m × 0.2 mm I.D., 0.33 μm film thickness (Hewlett-Packard, Avondale, PA, USA) was used under the following conditions: oven temperature programme, 2 min at 160°C, 20°C/min to 250°C, 1 min at 250°C, 20°C/min to 290°C and finally 3 min at 290°C; carrier gas, helium at a flow-rate of 1 ml/min; ion source temperature, 250°C; GC–MS interface maintained at 290°C.

The ionization was initiated with 40-eV electrons with an emmission current of 250 μA. To perform mass fragmentography the ions *m/z* 216.0423 for 8-MOP and 228.0786 for TMP were recorded with a dwell time of 0.17 s.

The areas of the mass fragmentographic peaks were integrated using the data system with operator selection of the baseline points.

RESULTS AND DISCUSSION

This method involves simplified two-step extraction of the sample. This is quick and effective, and many samples can be extracted in a short time. The analytical recovery of the drug was assayed at different concentrations of 8-MOP, ranging from 1 to 500 ng/ml, added to a pool of plasma and to a saline solution. The recovery, obtained by comparision of the results from the analysis of the saline and plasma solutions, ranged from 90% to 95%. However, to rule out variations due to different extraction efficiences,

an internal standard was used. We chose TMP because its behaviour is similar to that of 8-MOP during extraction [13]. This procedure has also been successfully used in the analysis of the samples by HPLC (data not shown).

The mass fragmentograms obtained after injection of the extracted samples do not contain any interference with the psoralen peaks. The following commonly co-administered drugs do not interfere with the analysis: cyclosporine, azothiaprine, methylprednisolone, nifedipine, calcitriol, calcium carbonate, athenolol, captopril.

A typical mass fragmentogram of a sample prepared as described above is shown in Fig. 1. The retention times were 7.91 min for 8-MOP and 8.77 min for TMP.

The calibration curve shows a linear relationship between the peak areas of 8-MOP and TMP over a wide range of 8-MOP concentrations (1–500 ng/ml). The regression equation yields a straight line that goes through the origin: $y = 0.74x$, where y is the 8-MOP concentration (ng/ml) and x is the peak-area ratio of 8-MOP to TMP. The correlation coefficient is 0.99999. The standard error of the slope is $2.47 \cdot 10^{-2}$.

We estimated precision of the assays by mea-

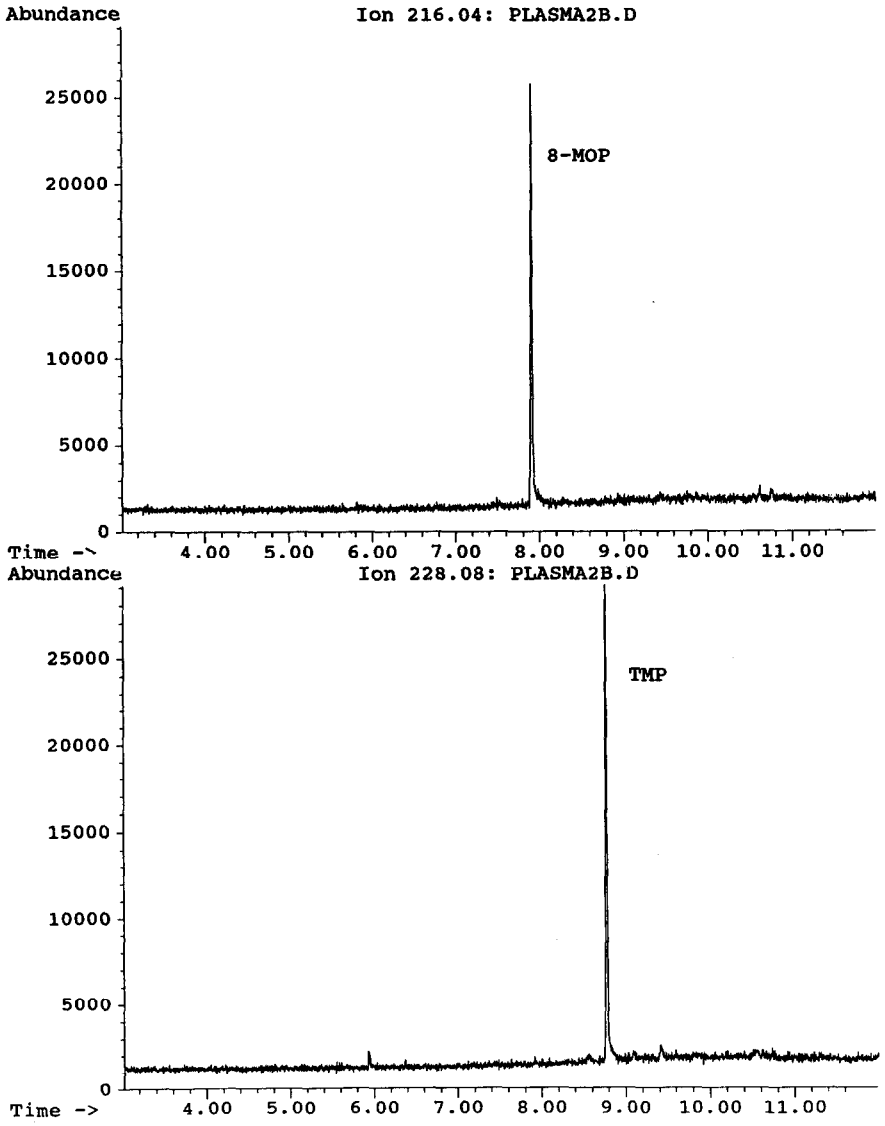

Fig. 1. Mass fragmentogram of a sample processed as described. The upper and the lower lines show the TMP and the 8-MOP peaks, respectively. Time in min.

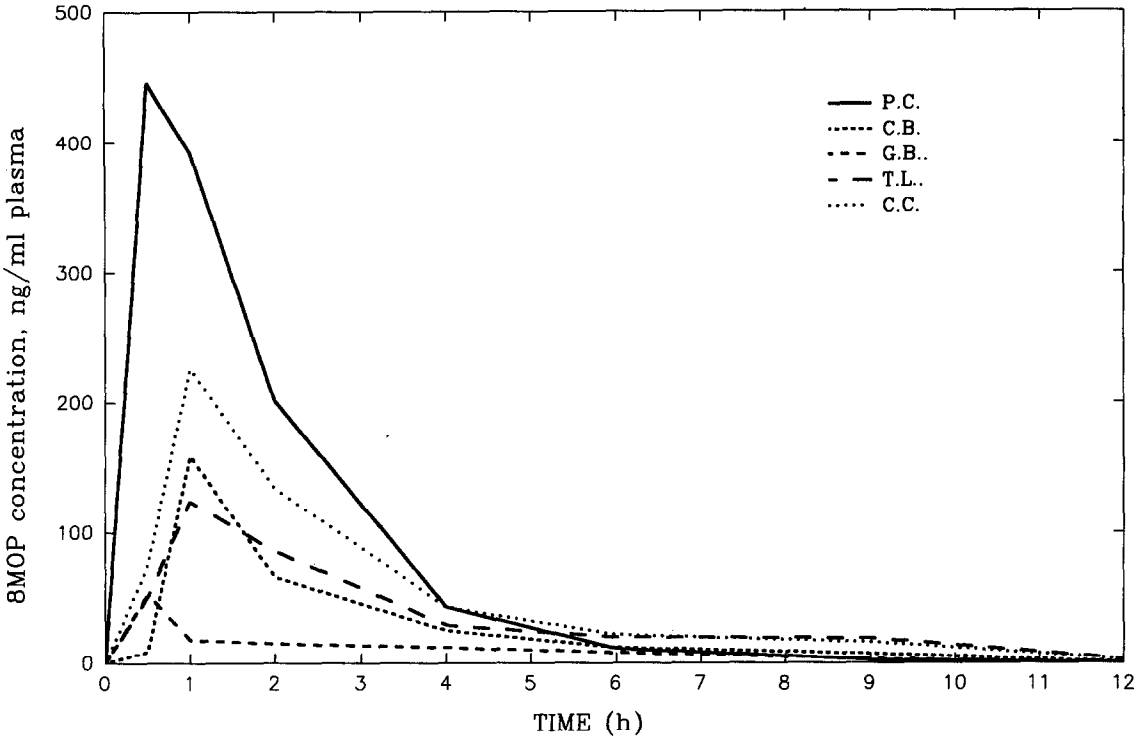

Fig. 2. Absorption curves for 8-MOP after oral administration of 0.6 mg/kg to five patients undergoing photopheresis.

suring five samples of pooled plasma supplemented with 8-MOP over the concentration range 1–500 ng/ml. The within-run precision was assessed by analysing ten replicate samples in the same run (mean C.V. 1.186%). The between-run precision was determined by analysing the same control samples for ten consecutive working days (mean C.V. 1.362%) (Table I).

At a signal-to-noise ratio of 3, the detection limit for 8-MOP was 40 pg/ml.

TABLE I

PRECISION OF THE DETERMINATION OF 8-METHOXYPSORALEN IN PLASMA

Concentration (ng/ml)	Coefficient of variation (%)	
	Within-run	Between-run
1	1.41	1.86
10	1.33	1.31
100	1.21	1.52
200	1.16	0.81
500	0.82	1.3

The absorption kinetics for 8-MOP, after ingestion of 0.6 mg/kg of the drug in five subjects (Fig. 2), show a wide interindividual variation. In

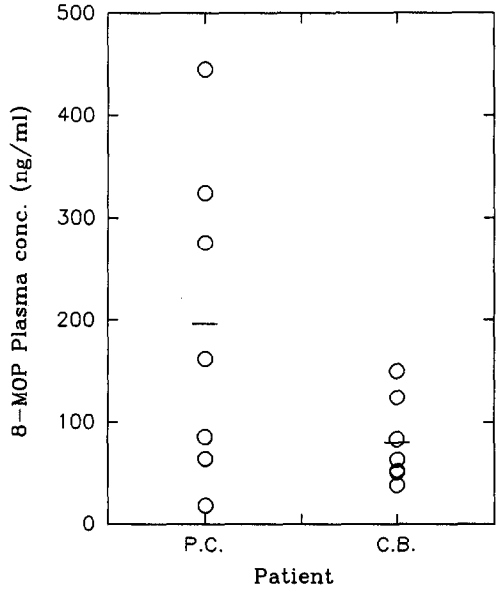

Fig. 3. Plasma levels of 8-MOP in two patients 30 min after drug ingestion. Horizontal bars indicate the mean.

addition, repeated 8-MOP determinations at monthly intervals in the blood of two patients, collected 30 min after the ingestion, illustrate an important intraindividual variation in the psoralen absorption, often with a suboptimal blood level of the drug (Fig. 3). The obvious consequence of these observations is that the psoralen level has to be strictly monitored because the efficacy of the therapy depends on the 8-MOP and UVA doses [4].

The GC–MS procedure described here could be used for the routine assay of 8-MOP levels in subjects undergoing 8-MOP and UVA photochemotherapies. In addition, it is very sensitive and precise even at very low concentrations, and consequently a useful tool for measuring psoralen levels in other biological fluids as well.

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REFERENCES

1 J. A. Parrish, T. B. Fitzpatrick, L. Tannenbaum and M. A. Pathak, *N. Engl. J. Med.*, 29 (1974) 1207.
2 R. L. Edelson, C. L. Berger, F. P. Gasparro, K. Lee and J. Taylor, *N. Engl. J. Med.*, 316 (1987) 297.
3 A. H. Rook, B. V. Jegasothy and P. Heald, *Ann. Intern. Med.*, 112 (1990) 303.
4 F. P. Gasparro, R. Dall'Amico, D. Goldminz, E. Simmons and D. Weingold, *Yale J. Biol. Med.*, 62 (1989) 579.
5 C. V. Puglisi, J. A. F. De Silva and J. C. Meyer, *Anal. Lett.*, 10 (1977) 39.
6 L. M. Stolk, R. De Ruiter, A. Saadawi, A. H. Siddiqui and R. H. Cormane, *J. Chromatogr.*, 423 (1987) 383.
7 L. M. Stolk, A. H. Siddiqui and R. H. Cormane, *Br. J. Dermatol.*, 104 (1981) 443.
8 B. Ljunggren, D. M. Carter, J. Albert and T. Reid, *J. Invest. Dermatol.*, 74 (1980) 59.
9 F. P. Gasparro, J. Battista, J. Song and R. L. Edelson, *J. Invest. Dermatol.*, 90 (1988) 234.
10 C. H. Ketchum, A. Robinson and S. T. Huang, *Clin. Chem.*, 36 (1990) 1956.
11 T. Fischer, P. Hartvig and U. Bondesson, *Acta Dermatovener (Stockholm)*, 60 (1979) 177.
12 J. Taskinen, N. Vahvelainen and P. Nore, *Biomed. Mass. Spectrom.*, 7 (1980) 556.
13 T. J. Sullivan, J. L. Walter, R. F. Kouba and D. C. Maiwald, *Arch. Dermatol.*, 122 (1986) 768.