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Note

Rapid, sensitive gas chromatographic analysis of 8-methoxypsoralen in human plasma

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8-Methoxypsoralen (8-MOP) is increasingly used in the treatment of psoriasis. Quantitative analysis of 8-MOP in biological fluids has been performed by measurement of the radioactivity of the drug in combination with thin-layer chromatography (TLC) [1]. A spectrophotometric method employing 5 ml of plasma has been described [2]. A sensitive, simple high-performance liquid chromatographic (HPLC) technique using 4 ml of plasma [3] and gas—liquid chromatographic (GLC) methods [4, 5] with a flame-ionization detector (FID), including TLC clean-up of extracts from 2—10 ml plasma have been described in the literature for quantifying 8-MOP levels. Unfortunately these methods are time-consuming. Recently a determination of 8-MOP by electron-capture gas chromatography was described [6]. However, this method requires plasma volumes of 2 ml and a lengthy extraction procedure. The aim of this study therefore was to develop a very rapid, simple method for determining 8-MOP in small plasma samples of 0.1—0.5 ml.

EXPERIMENTAL

Reagents and chemicals

8-MOP and the internal standard 5,8-dimethoxypsoralen (Fig. 1) were of analytical grade. ^{14}C-labelled 8-MOP (specific radioactivity 2 μCi/mg) was synthesized in the isotope laboratory of the Biochemical Department of the Dr. Karl Thomae GmbH. It was labelled at the methoxy-position. The toluene used was from Mallinckrodt (Wesel, G.F.R.), nanograde quality, No. 8092; the hydrochloric acid was purchased from Merck (Darmstadt, G.F.R.), p.a. quality, No. 319.

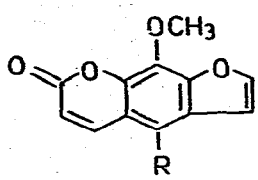

Fig. 1. Structural formulae of furocoumarins: 8-MOP = 8-methoxypsoralen; internal standard, IS = 5,8-dimethoxypsoralen.

Apparatus

The gas chromatograph used was a Perkin-Elmer F 22 equipped with a ^{63}Ni electron-capture detector (ECD) with pulse-frequency modulation. A glass column (2 m × 2 mm I.D.) was used, filled with Chromsorb 750, (80—100 mesh; Pierce, Rockford, Ill., U.S.A.), coated with 3% OV-17 (Perkin-Elmer, Überlingen, G.F.R.). The column was conditioned at 280° for three days, silanization was effected with Silyl-8 (Pierce). Operating conditions were: column-temperature, 220°; injector-temperature, 230°; detector-temperature, 250°; carrier gas, argon—methane (95:5). The integrator was a Hewlett-Packard 3380 S.

Analytical procedure

The blood was sampled via Braunülen® (Braun, Melsungen, G.F.R.) heparinized and centrifuged in glass tubes. The plasma was pipetted-off and stored frozen. It was thawed at room temperature, and 0.5-ml portions were pipetted into 10-ml glass-stoppered test-tubes, containing 0.5 ml of 0.2 *N* HCl and 2 ml toluene (containing 200 ng/ml internal standard), and mixed 15 min on a shaking-machine. After centrifuging, the plasma-phase was frozen at -20°, the organic phase transferred into another tube and evaporated to about 200 µl in a thermoblock at 40° under a gentle stream of nitrogen. If the tubes were brought to dryness, the residue was reconstituted in 200 µl of toluene. From each of those samples 4 µl were injected into the gas chromatograph. The calibration curve was constructed by adding 50—500 ng 8-MOP (dissolved in 0.1 ml methanol) to plasma.

RESULTS AND DISCUSSION

The use of an ECD [6, 7] instead of an FID [4], produces an increase in the sensitivity and selectivity. This means that the volume of the sample can be reduced from 0.5 ml to 50 µl if suitably small tubes are used.

To avoid interfering peaks in the chromatograms, Ehrson et al. [6] used a clean-up procedure which takes advantage of the ring opening of 8-MOP at the lactone position. This procedure is time consuming. If the extraction is performed from an acidic medium, the blanks are much lower, as is shown in Fig. 2. These blanks correspond to a concentration of only 5—10 ng/ml. We analysed about 30 different human plasma samples and found this interference to be constant for these individuals. We have strong evidence, that there is no ring-opening of 8-MOP in plasma which could be re-lactonised during our acidic extraction procedure:

Firstly, plasma from a patient who had received 40 mg 8-MOP, was extracted

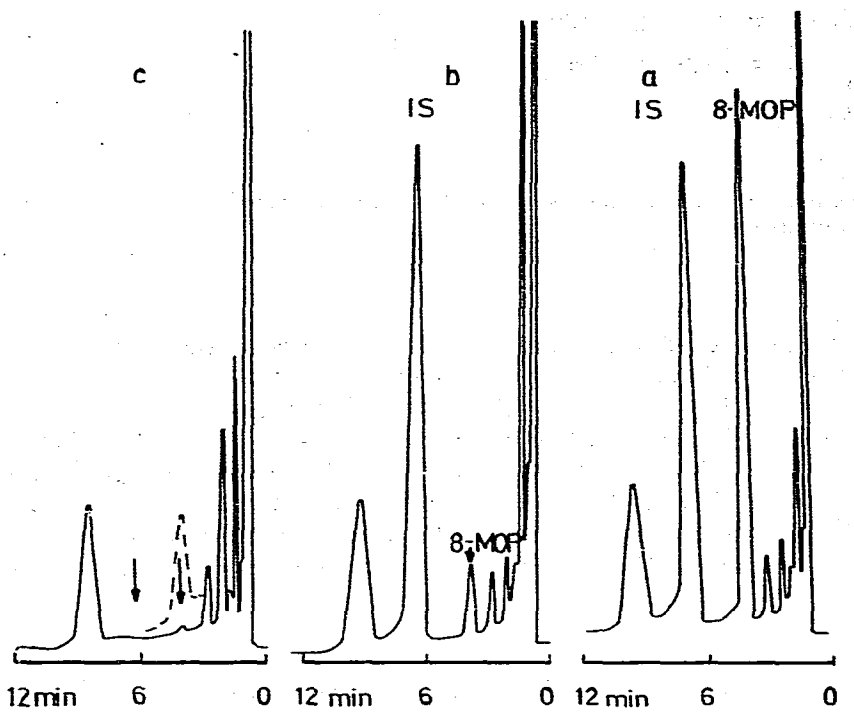

Fig. 2. Chromatograms of 8-MOP showing human plasma at a level of: (a) 500 ng/ml; (b) 50 ng/ml; (c) blank. The dotted line in (c) shows the blank for extraction at neutral pH.

at a neutral pH with toluene to prevent lactonisation and then the organic phase was washed with hydrochloric acid. With this method the plasma level was the same as that measured after our direct extraction method.

Secondly, during our metabolic studies [1] we were able to demonstrate (by structure elucidation of urine metabolites) that there is only an insignificant ring cleavage at the lactone-position of 8-MOP in man.

Thirdly, Ehrson [6] could not detect this open lactone molecule by ion-pair extraction, even at low levels.

By liquid scintillation counting, the recovery of ^{14}C-labelled 8-MOP was 98.6 ± 0.9% for n = 5 at a concentration of about 900 ng/ml. With GLC (using the method described for the construction of the calibration curve) the recovery was at a level of 100 ng/ml 8-MOP 100 ± 2.6% for n = 5 and at a level of 500 ng/ml 8-MOP 96.9 ± 1.8%, also for n = 5.

The calibration curve is linear, between 50—500 ng/ml 8-MOP. This range is attained with therapeutic doses. The reproducibility over a period of one day was studied in a concentration range of 50—500 ng/ml 8-MOP. The reproducibility over several days was studied by preparing 15 plasma samples containing 500 ng/ml 8-MOP and analysing them on different days. The results of these experiments show a good reproducibility (Table I).

However, there are three points that have to be taken into consideration to obtain good results. Firstly, some types of plastic material give rise to interfering peaks in the gas chromatogram. We therefore used Braunülen for collecting the blood and centrifuged it in glass tubes. A further point of consideration

TABLE I

REPRODUCIBILITY OF 8-MOP DETERMINATION ON DAY (A) AND BETWEEN DAYS (B)

	Plasma level (ng/ml)	Mean peak area ratio	No. of determinations	S.D. (%)
(A)	50	0.076	4	4.3
	100	0.149	4	2.0
	200	0.285	4	0.9
	300	0.412	4	1.8
	400	0.527	4	2.2
	500	0.654	4	2.1
(B)	500	0.650	15	2.4

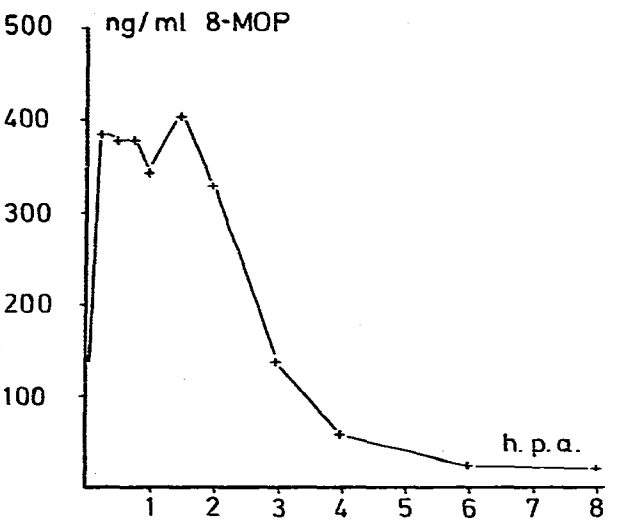

Fig. 3. Plasma concentration of 8-MOP in a human subject following p.o. administration of 40 mg 8-MOP as a solution.

is the evaporation step performed in the thermoblock. Care must be taken that the tubes are taken off the thermoblock as soon as the evaporation is complete, to prevent the observed loss of 8-MOP. Finally, a new gas chromatographic column shows some tailing. This can be reduced by injecting plasma extracts containing 8-MOP at a level of 500 ng/ml.

Fig. 3 shows the plasma concentration of 8-MOP in a human subject following p.o. administration of 40 mg 8-MOP in a solution. This plasma level is in good agreement with HPLC studies [3] and with TLC studies [1].

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