Analysis of Methoxsalen in Plasma by Reversed-Phase High-Performance Liquid Chromatography

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Abstract □ A high-performance liquid chromatographic method for the determination of methoxsalen in plasma was developed. The method includes extraction from plasma of the drug and the internal standard (5-methoxypsoralen) into methylene chloride. Chromatography was performed on a reversed-phase C_8 column connected with a UV detector set at 254 nm. The mobile phase was methanol–acetonitrile–water (2:30:68). For a plasma sample of 0.25 ml, the maximal sensitivity was ~10 ng/ml. Accuracy was within 7.5% for therapeutic plasma levels, and the coefficient of variation varied between 4.3 and 0.9% for 28 and 300 ng/ml of plasma, respectively.

Keyphrases □ Methoxsalen—high-performance liquid chromatographic analysis, human plasma □ High-performance liquid chromatography—methoxsalen, human plasma □ Photosensitizer—methoxsalen, high-performance liquid chromatographic analysis, human plasma

Methoxsalen (8-methoxypsoralen), a furocoumarinic derivative, is used in conjunction with phototherapy in the treatment of psoriasis (1). Different methods of analysis of methoxsalen in plasma have been published. Fluorodensitometric methods (2, 3) are time consuming, and flame-ionization GLC (4) is not sensitive enough for measuring therapeutic plasma levels. Electron-capture GLC (5–7) and GLC–mass spectrometry (8) also have been described.

Since methoxsalen has a very high UV absorbance, a high-performance liquid chromatographic (HPLC) method with UV detection was developed. HPLC using a silica column also has been used (9, 10). HPLC on a reversed-phase column[1] was proposed (11), with the internal standard griseofulvin being added after the extraction. Reversed-phase HPLC on a C_{18} column with trimethylpsoralen as the internal standard also was investigated (8, 12).

This report describes an HPLC method with a simple and rapid extraction procedure using a C_8 column and 5-methoxypsoralen as the internal standard.

EXPERIMENTAL

Reagents and Solvents—Methoxsalen[2] (8-methoxypsoralen) and 5-methoxypsoralen[3] were used as received. Methylene chloride[4], methanol[5], and acetonitrile[5] were also used.

Equipment—A microprocessor-controlled high-performance liquid chromatograph[6] with a fixed-wavelength UV detector[7] (254 nm), an automatic injector, and a 10-μl sample loop were used.

Separations were performed on a reversed-phase column[8] (250 × 4.6 mm i.d.). The column was operated at 40°. Chromatograms were traced on a printer/plotter[6] at a chart speed of 0.5 cm/min.

Chromatographic Conditions—The mobile phase, methanol–acetonitrile–water (2:30:68), was pumped through the column at a flow rate of 1.5 ml/min at 500–1000 psi.

Procedure—To 250 μl of plasma in a 5-ml glass conical tube was added 200 ng of the internal standard, 5-methoxypsoralen (20 μl of a 0.001% methanol solution). The sample was extracted with 700 μl of methylene chloride for 30 sec on a mixer[9]. After centrifugation at 5000 rpm at 4° for 10 min, the organic phase was transferred to another 5-ml glass conical tube and evaporated to dryness at room temperature under nitrogen. The residue was stored at −20° until analysis. The extract was reconstituted in 20 μl of methanol just prior to chromatography, and 10 μl was injected.

Absolute Recovery—Absolute recovery of methoxsalen from plasma was determined by adding the internal standard after extraction and comparing peak height ratios with peak height ratios of a calibration curve. This calibration curve was obtained by injecting methanol solu-

Table I—Absolute Recovery of Methoxsalen from Plasma [a]

Quantity Added to 250 μl of Plasma, ng	Mean, %	Coefficient of Variation, %
7.0	92.8	6.6
36.0	87.2	8.8
50.0	87.4	5.6

[a] $n = 5$.

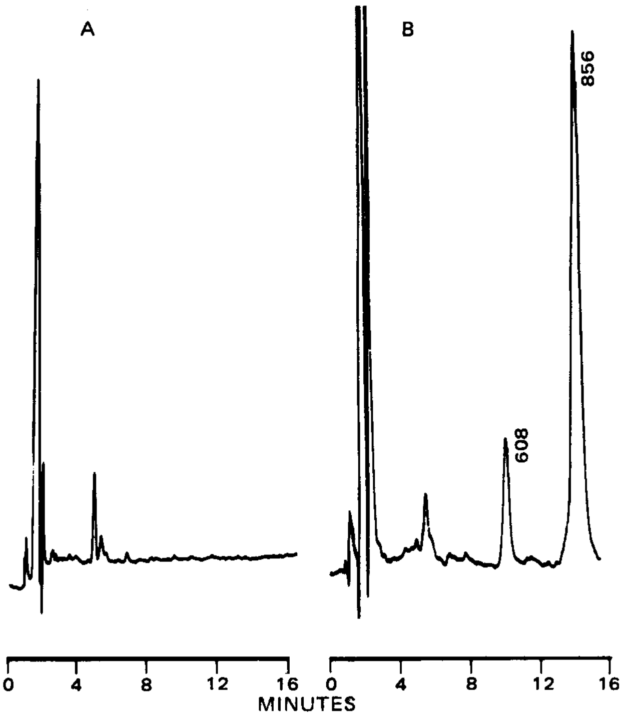

Figure 1—*Chromatogram of a 250-μl extract of human blank plasma (A) and of a 250-μl extract of human blank plasma spiked with 25 ng of methoxsalen* (R_t *608 sec) and 200 ng of the internal standard* (R_t *856 sec) (B).*

[1] μBondapak CN.
[2] Covor, Brussels, Belgium.
[3] Promedica, Paris, France.
[4] Pro analysis grade, Merck, Darmstadt, West Germany.
[5] Lichrosolv grade, Merck, Darmstadt, West Germany.
[6] Spectra Physics SP-8000, Eindhoven, The Netherlands.
[7] Spectra Physics SP-8200, Eindhoven, The Netherlands.
[8] Lichrosorb 10 RP 8, Chrompack, Merksem, Belgium.
[9] Vortex, Cenco Instruments Mij N. V., Breda, The Netherlands.

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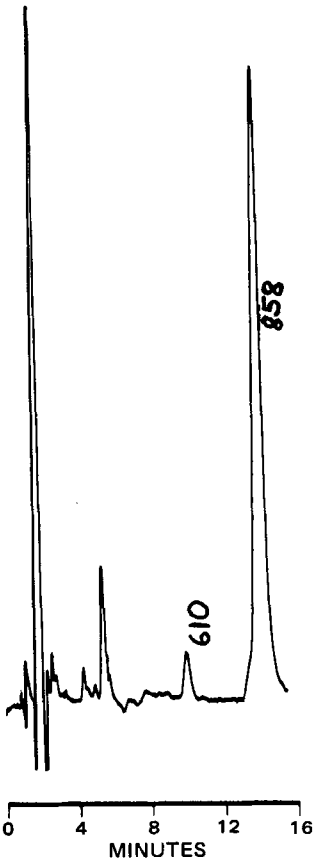

Figure 2—*Chromatogram of a 250-μl extract of plasma of a patient after oral intake of 30 mg of methoxsalen (R_t 610 sec); the sample was spiked with 200 ng of the internal standard (R_t 858 sec).*

tions containing 10–80 ng of methoxsalen and 100 ng of the internal standard.

RESULTS AND DISCUSSION

The extraction gave good recoveries (87–93%) of methoxsalen from plasma (Table I).

Plasma standard curves were linear for concentrations ranging from 12 to 1000 ng/ml. The average slope for 16 standard curves, assayed over 5 months, was 0.008436 ± 0.000422 (mean ± *SD*), with an average correlation coefficient of 0.9980 ± 0.0023 (mean ± *SD*). For a plasma sample of 0.25 ml, the maximum sensitivity was ~10 ng/ml.

Within-run accuracy and precision were acceptable (Table II). For testing of the between-run reproducibility and accuracy, aliquots were taken 16 times over a 5-month period from a pool containing 100.0 ng of methoxsalen/ml of plasma; the average result was 102.4 ± 5.2 ng/ml (mean ± *SD*) (n = 16).

Table II—Within-Run Precision and Accuracy of Methoxsalen Assay

Quantity Added to 250 μl of Plasma, ng	Relative Error, %	Coefficient of Variation, %
7 (n = 4)	+7.5	4.3
36 (n = 5)	+2.7	5.0
50 (n = 5)	−2.9	2.6
75 (n = 5)	−3.2	0.9

Figure 1A shows a representative chromatogram of an extract of human blank plasma. Figure 1B shows a chromatogram of human blank plasma spiked with methoxsalen and the internal standard. Both products were well resolved. Figure 2 shows a chromatogram of a plasma extract of a patient 130 min after the intake of 30 mg of methoxsalen, spiked with the internal standard.

The proposed method is suitable for plasma sample analysis for pharmacokinetic studies of methoxsalen in animals and humans. The extraction is simple and rapid, and only 700 μl of the extraction solvent is needed. This procedure is an improvement in comparison to other methods (8–12). The small volume of plasma used (250 μl) enables repeated sampling.

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