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Short communication

High-performance thin-layer chromatographic determination of 5-methoxypsoralen in serum from patients

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Abstract

A simple and rapid high-performance thin-layer chromatographic (HPTLC) determination of 5-methoxypsoralen in serum is necessary for the therapeutic survey of patients treated with Puvatherapy (psoralen+UV A). The assay for this biological fluid involves an extraction with heptane–dichloromethane (4:1, v/v). The analytical method is linear from 50 to 250 ng/ml. This assay range is adequate for analysing human serum, as it corresponds to psoralen concentrations measured in serum from patients treated with psoralen and UV A against psoriasis and vitiligo. The limit of detection is 15 ng/ml. The coefficient of variation was less than 7%. © 1997 Elsevier Science B.V.

Keywords: 5-Methoxypsoralen

1. Introduction

Psoralens are furocoumarins, used for the treatment of dermatological disease [1–4]. Up until now, psoralen in the serum of psoriatic patients has been determined by high-performance liquid chromatography (HPLC) [5], which is the most used technique for psoralen assessment in serum because of its high sensitivity and specificity. Nevertheless, this method requires a long analysis time. This paper describes a high-performance thin-layer chromatography (HPTLC) method that allows the simultaneous analysis of several serum samples.

2. Experimental

2.1. Reagents

All chemicals were of the analytical grade. The following substances were used: 5-methoxypsoralen (5-MOP), trimethoxypsoralen (TMP) (Bergaderm, Paris, France), methanol (Carlo Erba, Rueil Malmaison, France), heptane and dichloromethane (Merck, Nogent sur Marne, France).

2.2. Sample preparation

The psoralens were extracted from solvent according to the Stolk method [6]. To 1 ml of serum, 5 ml of heptane–dichloromethane (4:1, v/v) were added. After vortex-mixing for 7 min and centrifugation at

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5500 *g* for 2 min, 4 ml of the organic layer were transferred to a clean glass tube and evaporated to dryness at 50°C under a stream of nitrogen. The residue was dissolved in 40 μl of methanol under vortex-mixing for 40 s. A 3-μl volume was used for spotting.

2.3. Materials

Stationary phase: HPTLC plates RP_{18}, (10×10 cm; ref. 13724) from Merck (Darmstadt, Germany) were used [7]. An automatic TLC sampler III (ATS III) Camag (Merck, Strasbourg, France) was used for spotting. HPTLC plate development was performed in a Camag HPTLC vario chamber from a (4:1, v/v) methanol–water solvent [7]. Plates were scanned in fluorescence mode (excitation, 313 nm; emission, 490 nm) with a TLC densitometer scanner II Camag.

2.4. Assay validation

No demixing problems were observed and the results were reproducible. The standard curves for serum were obtained by adding 5-MOP to drug-free biological fluids to obtain a concentration of 25–250 ng/ml, and TMP was added as an internal standard [8,9] at 100 ng/ml. No blanks were included in these standard curves. Standard serums were prepared under operating conditions as described above. Accuracy and precision of the 5-MOP assay in the serum were determined by analysing eight replicates for each of six concentrations (25, 50, 100, 150, 200 and 250 ng/ml).

3. Results and discussion

The chromatogram shown in Fig. 1 is of the serum from eight patients after 5-MOP and TMP extraction. At the starting spots, there was always residual fluorescence. These "impurities" could arise from serum extraction (Fig. 1).

The calibration curve for 5-MOP was linear over the concentration range of 50 to 250 ng/ml. This range corresponds to psoralen concentrations measured in serum from patients treated with PUVA (psoralen+UVA) against psoriasis and vitiligo. Below 50 ng/ml, the method was non-linear (Table 1). The equation determined from six different concentrations (experiments repeated eight times) was $y=-53.238\cdot10^{-3}+7.184\cdot10^{-3}x$ ($r^2=0.983$), where y is the peak-area ratio of 5-MOP/TMP, x is the concentration of 5-MOP (in ng/ml) and r is the correlation coefficient. As usual, the detection limit, 15 ng/ml, was determined with the peak area equal to three times the background and the quantification limit was considered to be 10/3 of the detection limit. We determined only the response, y, the inter-day precision (expressed as a coefficient of variation, C.V.) and the inter-day accuracy of the 5-MOP assays in serum (Table 1).

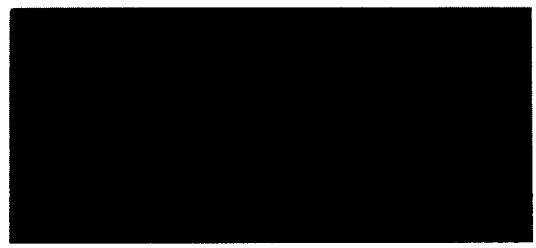

Fig. 1. Chromatogram of eight different serum samples. (A) Migration level of TMP; (B) migration level of 5-MOP; (C) migration level of the solvent (methanol–water); (D) migration level of an impurity of the serum; (E) starting position of the spot.

The coefficients of variation were less than 7% ($n=8$) (3% in HPLC) [5] over the concentration range 50–250 ng/ml. The accuracy lies between 97.5 and 107.2%. Pharmacokinetic analysis of serum from patients after oral intake of 5-MOP (1.2 mg/kg body weight) was carried on in respect of the calibration curve. The results are similar to those obtained by HPLC [5]. The worst difference between

Table 1
Inter-day precision and accuracy ($n=8$)

Spiked concentration (ng/ml)	Response (y)	C.V. (%)	Accuracy (%)
25[a]	0.12	11.28	72.84
50	0.33	2.52	99.78
100	0.67	3.16	100.00
150	0.98	2.23	97.55
200	1.33	6.88	99.40
250	1.80	4.36	107.25

[a]The concentration of 25 ng/ml is not in the linear range, which explains the bad values obtained for C.V. and accuracy.

5-MOP concentrations obtained using these two methods was 13%.

In summary, this newly developed HPTLC method for 5-MOP is highly sensitive and selective and has good reproducibility. It can be used to rapidly measure concentrations of 5-MOP at trace levels in serum for the optimal clinical management of patients. The simple HPTLC method enables, in a limited time, therapeutic drug monitoring to be performed (for the simultaneous analysis of between eight and twelve samples, the total analysis time required was a maximum of 30 min). This is not the case with HPLC, where analysis takes 10 min for a single sample.

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