NOTE

PURIFICATION OF [^{3}H]-8-METHOXYPSORALEN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A sample of [^{3}H]-8-methoxypsoralen was purified by high performance liquid chromatography on silica gel.

KEY WORDS

[^{3}H]-8-methoxypsoralen, 8-MOP, high performance liquid chromatography, HPLC.

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8-Methoxypsoralen (8-MOP), in combination with long wave ultraviolet light (UVA) is widely used during the last few years for the treatment of psoriasis, one of the most common skin diseases (1,2). Penetration, resorption, metabolism and distribution of 8-MOP in laboratory animals was studied using tritiated 8-MOP; the purity of the tritiated compound was usually checked by thin layer chromatography (TLC) and scanning (3,4,5).

[G-^{3}H]-8-MOP was purchased from The Radiochemical Centre, Amersham, U.K. to study the fate of 8-MOP in rats. The drug was labelled by exchange with tritiated water and was purified by TLC on silica gel (original radiochemical purity 98 %). The sample of [G-^{3}H]-8-MOP obtained was custom prepared more than two years ago; it contained 4.40 mCi (3.66 mg) of tritiated 8-MOP (specific activity 260 mCi/mmol) in 4.7 ml of toluene and had been stored at -20°C. Therefore, we had to recheck its radiochemical purity and also to purify the compound, if necessary.

TLC on silica gel (together with carrier 8-MOP) using chloroform : petroleum ether (bp 40-60°C) 1:1 and scanning with a Berthold scanner showed three impurities : a non-migrating spot (3.6 %) and two other decomposition products (0.7 % each). The purity of the [^{3}H]-8-MOP was therefore estimated as 95 %.

Besides TLC, high performance liquid chromatography (HPLC) was also used for the analysis of the labelled drug. Two µCi of the tritiated 8-MOP was injected on a 30 cm x 4 mm ID column filled with µ Bondapak silica gel (Waters Associates,

Inc., Milford, MA., USA) and eluted with methylene chloride : n-hexane 90:10 at 1.5 ml/min (retention time of 8-MOP was 18 min). Seventy fractions of 0.75 ml were collected in 20 ml vials; after standing overnight in a hood to evaporate the solvents, 10 ml of Lipoluma (Lumac, Schaesberg, The Netherlands) was added to each vial. Counting was performed in a Berthold liquid scintillation spectrometer. The HPLC system revealed more impurities than TLC . Three compounds were eluted before 8-MOP and four had a longer retention time. The radiochemical purity of the tritiated 8-MOP was estimated as 96.3 %; however, since the start peak (3.6 %), detected with TLC, was not eluted from the column, the real purity of the labelled drug was nearer to 92-93 %. We therefore decided to repurify the compound; since HPLC showed a higher resolving power than TLC, the former technique was used.

A preparative HPLC silica gel column was needed for the purification of the [^{3}H]-8-MOP. However, since it was likely that part of the polar radioactive impurities remained fixed on the column, relatively high cost commercially available packing materials were not used. We used inexpensive 15 µ TLC silica gel 60 H (Merck, Darmstadt, GFR); finer particles were removed by flotation in water. The remaining silica gel was first dried at 80°C and then reactivated at 120°C overnight. The silica gel (12 g) was then suspended in 60 ml of carbon tetrachloride and the slurry was packed in a 25 cm x 10 mm ID column with a Haskel pump, model DSTV-122, at 250 bar, using n-hexane as the pressurising liquid. The HPLC apparatus consisted of a Milton-Roy miniPump equipped with a home made pulse dampener, an Altex model 150 B biochemical UV monitor (254 nm) with an analytical

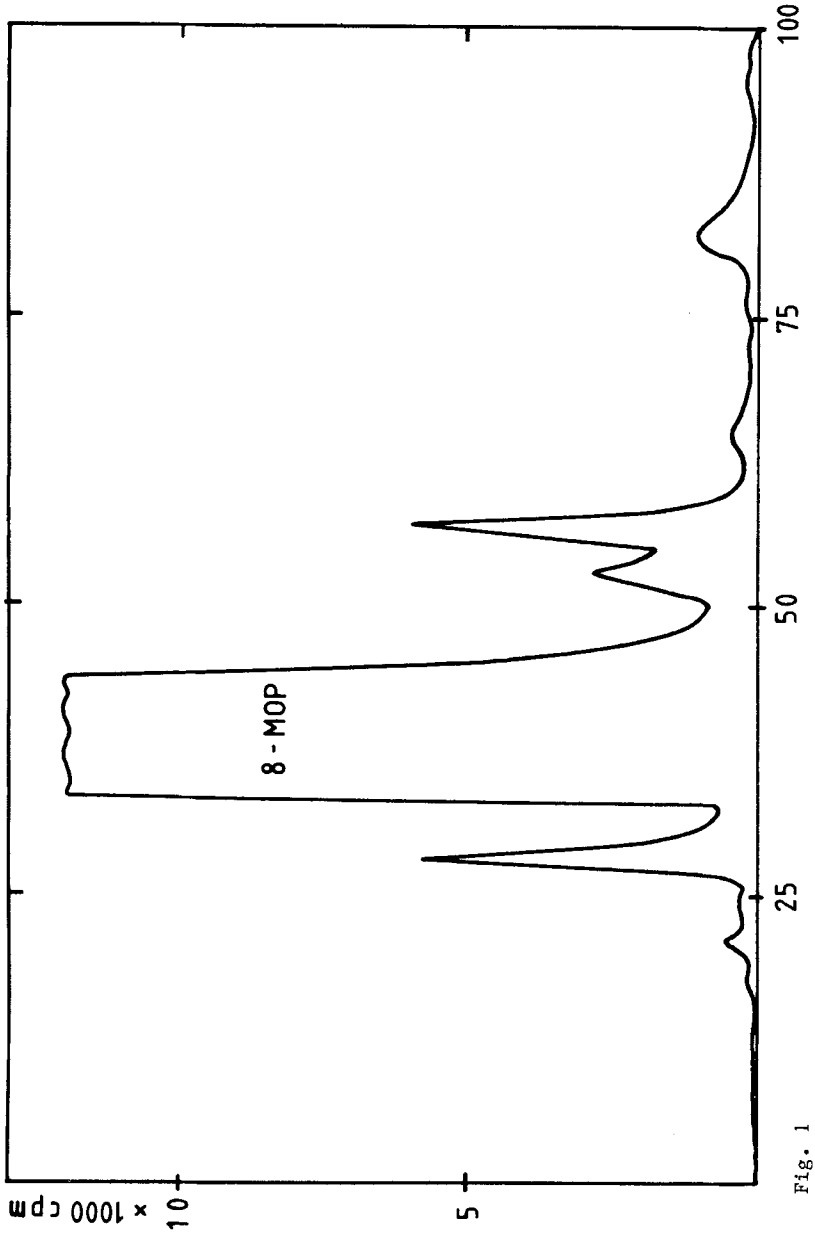

Fig. 1

Preparative high performance liquid chromatography of 1.6 mg of 8-methoxypsoralen spiked with 2.5 μCi of [^{3}H]-8-methoxypsoralen on a 25 cm X 10 mm ID column packed with 15 μ TLC silica gel 60H (Merck). Mobile phase : methylene chloride : n-hexane 60:40; flow rate 4 ml/min. Detection : 2 ml (0.5 min) fractions were collected, evaporated and counted in 10 ml of Lipoluma (Lumac).

cell and a Valco model CV-6-UHPa-N60 injector with a 0.5 ml loop (6). On this preparative column methylene chloride : n-hexane 60:40 had to be used at a flow rate of 4 ml/min, in order to obtain a retention time of 20 min for 8-MOP. The HPLC system was then tested with a mixture of 2.5 µCi of [^{3}H]-8-MOP and 1.6 mg of cold carrier 8-MOP. Hundred fractions of 2 ml were collected. Results are given in fig. 1; the resolution of the preparative HPLC system was comparable to that of the analytical column.

The preparative HPLC system was then used to purify the tritiated 8-MOP. The solution of the labelled compound was evaporated to dryness, leaving a yellow, oily residue; it was dissolved in a mixture of 0.4 ml of methylene chloride and 0.6 ml of n-hexane. Two 0.5 ml samples were injected. The 8-MOP fractions, as monitored by the UV detector, were collected and evaporated to dryness; the white crystalline compound was dissolved in toluene and further stored at -20°C. The inexpensive packing material was discarded after use. Radiochemical yield was 3725 µCi (85 %); however, since the original product was about 92-93 % pure, real recovery was 92 %. The purified drug was again examined, using TLC and HPLC. By TLC no spot could be detected at the start. A run on the analytical HPLC column showed a radiochemical purity of 99.5 %.

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