Analysis of 8-Methoxypsoralen by High-Performance Liquid Chromatography

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We report a simple and rapid procedure for assaying 8-methoxypsoralen (8-MOP) in plasma by high-performance liquid chromatography (HPLC). The standard curve for the assay is linear for 8-MOP from 15 to 500 μg/L (y = 0.002x − 0.01, r = 0.99) with a lower limit of detection of 1.5 μg/L. Intra-assay precision (CV) was 6.0% at the 100 μg/L concentration and 10.0% at 50 μg/L (n = 30 each). Interassay precision was 6.4% at 100 μg/L and 7.0% at 50 μg/L (n = 50 each). Extraction recovery of 8-MOP was 98%. Common antiarrhythmics, sedatives, and hypnotics were found not to interfere.

The use of photoactive drugs such as 8-methoxypsoralen (8-MOP; methoxsalen) in treating hyperproliferative skin disorders and cutaneous T-cell lymphoma has evolved during the last 20 years. In the cell, 8-MOP intercalates between the basepairs of DNA. Upon exposure to ultraviolet A light, 8-MOP is activated to a transiently excited state in which it can form photoadducts with the pyrimidine bases of DNA and thereby inhibit DNA replication.

The success of photochemotherapy depends, in part, upon achieving an 8-MOP concentration in plasma >65 μg/L at the time of exposure to ultraviolet A light. The peak plasma concentration achieved and the time to reach it depend on the formulation used and vary among individuals. Therefore, monitoring plasma concentrations at the time of treatment is important. High-performance liquid chromatography (HPLC) is the method by which 8-MOP is most frequently measured.

Previously reported HPLC methods have included lengthy extraction procedures, nonaqueous chromatographic conditions, or the use of radiolabeled 8-MOP for the verification of recovery (1–3). The method we have developed for the assay of 8-MOP involves solid-phase extraction columns and one reagent, thus simplifying the sample preparation. The internal standard, 5-methoxypsoralen (5-MOP; bergapten), is readily available from commercial sources.

Materials and Methods

Sample collection and storage. For routine monitoring, blood samples were collected into sodium heparin-containing tubes from the antecubital vein of patients 2 h after 8-MOP had been administered orally. The plasma was separated at room temperature and stored in polystyrene tubes at −20 °C until assayed.

Apparatus. We used an HPLC system consisting of an LC-6A pump, SPD-6AV spectrophotometric detector, SCL-6A system controller, and C-R3A Chromatopac data processor (all from Shimadzu Corp., Kyoto, Japan) and a 10-cm Spheri-5 RP-18 chromographic column (Applied Biosystems, Foster City, CA).

Reagents. Methanol and acetonitrile were HPLC-reagent grade (Burdick and Jackson, Muskegon, MI). Stock solutions (0.1 g/L) of 8-MOP and the internal standard 5-MOF (Sigma Chemical Co., St. Louis, MO) were prepared in HPLC-grade methanol and stored in brown glass bottles at 4 °C. For use as the extraction solution, the 5-MOP stock solution was diluted with methanol to give a final concentration of 500 μg/L.

Standards and controls. Stock 8-MOP solution was serially diluted with drug-free plasma to prepare standards ranging in concentration from 62.5 to 500 μg/L. Controls (8-MOP of 50 and 100 μg/L) were similarly prepared. All standards and controls were aliquoted and stored in polypropylene tubes at −20 °C.

Sample preparation. Specimens, standards, and controls were allowed to thaw at room temperature. We applied 0.5 mL of sample to 3.0-mL C18 Bond Elut solid-phase extraction columns (Analytichem International, Harbor City, CA) that had first been washed with 9.0 mL of methanol followed by 9.0 mL of water. After sample application, the column was washed with 9.0 mL of water. The eluate 8-MOP from the column with 0.5 mL of the methanol extraction solvent (which contains 5-MOP). The eluates were collected into glass tubes and vortex-mixed, and the tubes were capped. We injected 20 μL of the eluate onto the chromatographic column.

Chromatographic conditions. The mobile phase was a mixture of glass-distilled, de-ionized water; methanol; and acetonitrile (65/25/10, by vol). Before use, we filtered the mobile phase through a 0.45-μm (pore size) filter (Millipore Corp., Bedford, MA) and degassed it by sonication under reduced pressure for 10 min. The flow rate was 0.7 mL/min, the detection wavelength 300 nm, and the chart speed setting 2.5 mm/min. Attenuation of the integrator signal was set at 1 and the detector sensitivity was 0.001 A full-scale. All chromatography was performed at 25 °C.

Results and Discussion

Representative chromatograms of extracted samples are shown in Figure 1. The retention times for 8-MOP and 5-MOP are 5.0 and 8.5 min, respectively. An unidentified peak eluting at approximately 2.8 min has been observed intermittently in the chromatograms of standards, controls, and patients' samples.

Both 8-MOP and 5-MOP have been reported to degrade upon prolonged exposure to light (4). However, we have not seen any changes in the ultraviolet absorption spectra of either compound, dissolved in methanol, after exposure to room light for as long as one month. Absorption maxima

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1 mol of 8-MOP or 5-MOP has a mass of 216.18 g.
Simple Screening Tests for the Emergency Identification of Methanol and Ethylene Glycol in Poisoned Patients

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We have developed simple and rapid screening tests for methanol and ethylene glycol in plasma of poisoned patients, based on the Toxi-Lab alcohol screening test. The compounds are first converted to formaldehyde, which diffuses from the patient’s sample into a glass-fiber test sheet impregnated with Schiff’s formaldehyde detection reagent. Subsequent exposure of the test sheet to acid and heat distinguishes formaldehyde from acetalddehyde. We recommend that samples giving positive results be analyzed by another technique to confirm the identity and concentration of the poison for use in prognosis and treatment.

Additional Keyphrases: toxicology • microdiffusion test • colorimetry

Methanol and ethylene glycol are frequently ingested in self-poisoning attempts or added to drinks to produce intoxication. Both compounds have highly toxic metabolites and may cause severe metabolic acidosis, coma, and death; methanol ingestion may lead to irreversible blindness. Active treatment involves administering ethanol to block metabolism of the poison and, in more severe cases, hemo-