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Note

Reversed-phase high-performance liquid chromatography of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide and metabolites

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The dialkyltriazene 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC, DIC, dacarbazine) is useful in the treatment of Hodgkin's disease, soft tissue sarcoma and melanoma [1]. Preliminary reports have also suggested effectiveness in regional perfusion of the extremity in locally recurrent melanoma [2, 3]. DTIC is known to undergo metabolism by two major pathways. In the presence of liver microsomes, DTIC is demethylated to form 5-(3-methyl-1-triazeno)imidazole-4-carboxamide (MTIC, MIC), which rapidly yields an active methyl cation and 5-aminoimidazole-4-carboxamide (AIC) [4, 5]. In the presence of ultraviolet light, a complex process of photodecomposition occurs [6]. Among a variety of intermediates, 2-azahypoxanthine (2AH) is the metabolite seen primarily in clinical situations [2, 7—9].

Current methods to quantitate the above compounds in biological fluids using high-performance liquid chromatography (HPLC) require the use of two separate chromatographic systems. One technique uses acidic paired-ion exchange to elute DTIC and basic paired-ion exchange to elute AIC and 2AH [7]. Another uses a reversed-phase column for DTIC and 2AH and basic paired-ion exchange for AIC [8, 9]. This report describes the separation of all three components on a single, reversed-phase phenyl column.

EXPERIMENTAL

Standards

DTIC (Miles Labs., West Haven, CT, U.S.A.), 2AH (Miles), and AIC (Sigma, St. Louis, MO, U.S.A.) were prepared in aqueous solutions in concentrations ranging from 0.05 to 1000 μg/ml. The form of DTIC used (DTIC-Dome) was the citrate salt containing 16% (w/w) mannitol. All samples were kept

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on ice and protected from light in amber glass vials. Known amounts of the above compound were also prepared in human plasma from normal volunteer donors for calibration measurement of extraction efficiency.

Plasma extraction

Plasma (1 ml) was vortexed for 60 s with 5 ml methanol—chloroform (3:1), allowed to stand at 4°C for 15 min and centrifuged for 10 min at 4°C (1300 *g*). The supernatant was then injected directly onto the column.

Apparatus

The HPLC equipment consisted of two solvent delivery pumps (Waters, Model 6000A), an automatic injector (Waters, WISP 710B), a 30 cm × 3.9 mm column with 10-μm μBondapak phenyl silica-based packing (Waters), a plotter-integrator (Waters, Data Module 730) and a system controller (Waters, Model 720). Absorbance detection (Waters, Model 441) was carried out at 254 nm.

Chromatographic conditions

Initial conditions consisted of 0.1% (w/v) ammonium formate buffer, pH 5.5—methanol—water (90:5:5) at 2.0 ml/min. At 0.5 min, a 1-min linear gradient was used to change the composition to 40:30:30 (v/v) at a flow-rate of 2.0 ml/min. Run time was 9 min, with an equilibration delay of 4 min. Injection volume was 20—100 μl, depending on drug concentration. Buffer was prepared from ammonium hydroxide (Mallinckrodt, Paris, KY, U.S.A.) and 88% formic acid (Fisher). HPLC-grade methanol was obtained from Fisher.

RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram of standards in distilled water. The components AIC, 2AH and DTIC elute at 2.6, 3.1 and 6.8 min, respectively. The limit of detection was approximately 2 ng for each component. Response was found to be linear up to 50 μg with a correlation coefficient of 0.9996. With an automatic sample injector, the overall accuracy of the method was 5%.

A chromatogram of an extracted plasma sample from a patient before and after an intravenous dose of DTIC is given in Fig. 2. Good resolution from endogenous plasma components, which elute at 1.7—2.1 min, is seen. A slight shift on baseline at 4—5 min owing to the gradient does not interfere with quantitation. Extraction efficiency averaged 95, 97 and 92% for AIC, 2AH and DTIC, respectively. The minimum detectable plasma concentration was 50 ng/ml for each compound, which is comparable to other methods [7]. At this level, the signal-to-noise ratio is 10:1, although baseline shift is prominent (about 45% of full scale at 0.005 sensitivity).

The combination of a phenyl stationary phase and an ammonium formate buffer mobile phase is a versatile one. It was developed by Israel et al. [10] in the study of the pharmacokinetics of adriamycin and modified by our laboratory to analyze several compounds, including melphalan [11], 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and adriamycin. The pH was increased from 4.0 to 5.5 to achieve optimum separation of AIC and 2AH

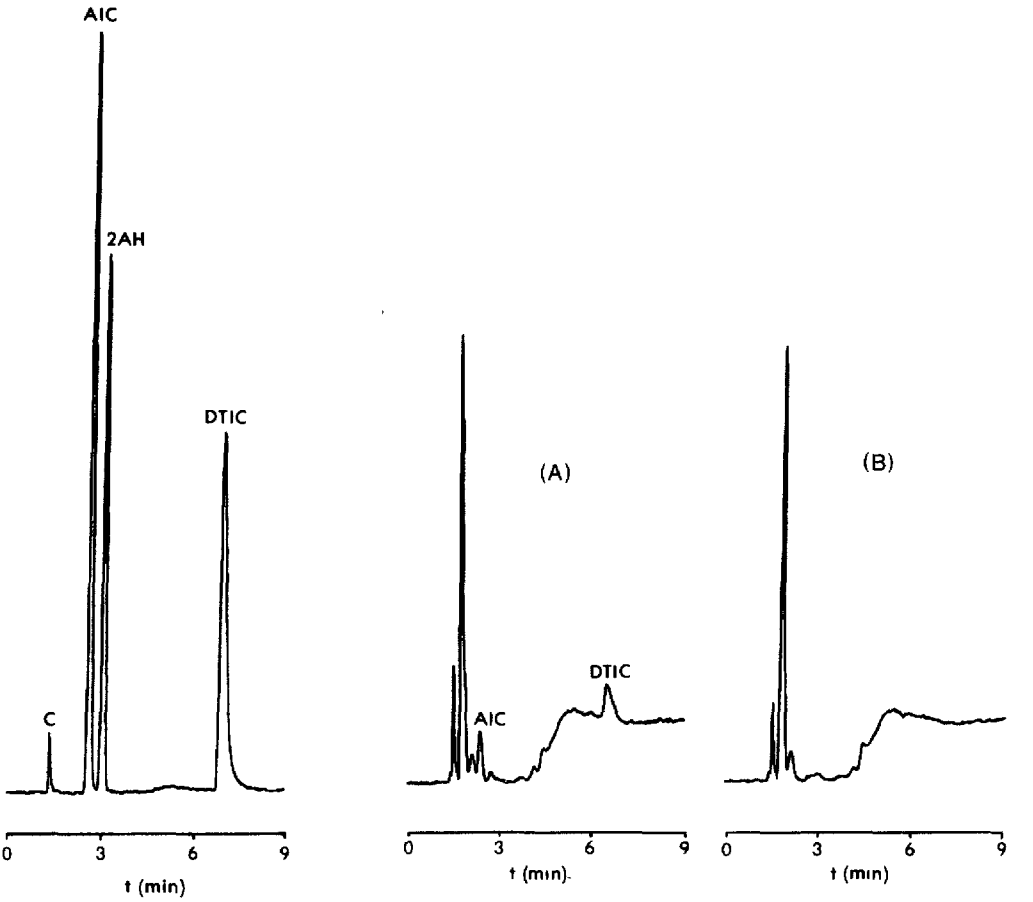

Fig. 1. HPLC profile of a standard mixture of approximately 100 μg/ml AIC, 2AH and DTIC in aqueous solution on a reversed-phase phenyl column (C = citric acid). Absorbance detector sensitivity was 0.2 at 254 nm. See text for experimental conditions.

Fig. 2. (A) HPLC profile of an extracted plasma sample from a patient 10 min after an 850 mg/m^2 intravenous infusion of DTIC over 1 h. Concentrations of AIC and DTIC are 4.9 and 13.4 μg/ml, respectively (injection volume, 20 μl). (B) HPLC profile of a pre-infusion sample from the same patient. Absorbance detector sensitivity was 0.02 at 254 nm.

from endogenous plasma components. Likewise, a methanol—chloroform (3:1) extraction resulted in a relatively clean sample with excellent recovery of the compounds of interest.

This paper presents a technique for quantitation of DTIC and metabolites which has the advantage of resolution of all components on a single column. Plasma extraction is efficient and gives a clean sample which preserves column life. We have used this method to study the pharmacokinetics of DTIC in plasma after systemic administration and in perfusate during regional perfusion of the extremity.

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