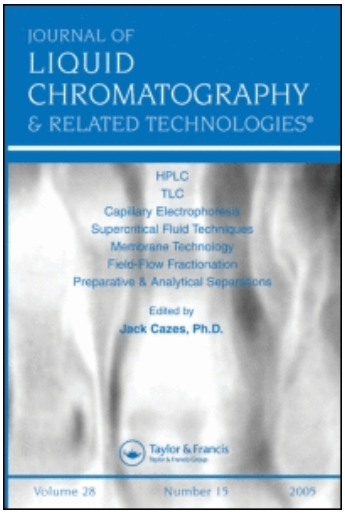

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Lu, K. , Savaraj, N. , Huang, M. T. , Moore, D. and Loo, T. L.(1982) 'High-Performance Liquid Chromatography (HPLC) of the New Antineoplastic 9,10-Anthracenedicarboxaldehyde Bis[(4,5-Dihydro-1 H-Imidazole-2-yl)hydrazone] dihydrochloride (CL 216,942; Bisantrene)', Journal of Liquid Chromatography & Related Technologies, 5: 7, 1323 — 1328
To link to this Article: DOI: 10.1080/01483918208067590
URL: http://dx.doi.org/10.1080/01483918208067590

JOURNAL OF LIQUID CHROMATOGRAPHY, 5(7), 1323-1328 (1982)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) OF THE NEW ANTINEOPLASTIC 9,10-ANTHRACENEDICARBOXALDEHYDE BIS[(4,5-DIHYDRO-1 H-IMIDAZOLE-2-YL) HYDRAZONE]DIHYDROCHLORIDE (CL 216,942; BISANTRENE)

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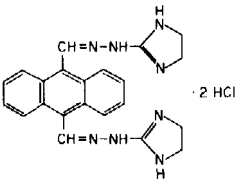
INTRODUCTION

9,10-anthracenedicarboxaldehyde bis[(4,5-dihydro-1H-imidazole-2-yl) hydrazone] dihydrochloride, (Bisantrene, CL 216,942, or NSC-337766) (Fig. 1) is a new anthracenedione derivative which has significant antitumor activity in a number of animal tumor systems including L1210 leukemia, P388 leukemia, Liberman plasma cell tumor, B16 melanoma, Ridgeway osteogenic sarcoma and colon tumor 26 in mice (1). Although structurally, it bears some resemblance to doxorubicin, bisantrene differs in producing less myocardial toxicity at equitoxic doses. Therefore, Bisantrene may be a useful antitumor agent in doxorubicin sensitive tumors (2). In order to study the pharmacokinetics of the agent in conjunction with the phase I and II clinical trial in our institute, we developed an analytical method for Bisantrene. The sampling and analytical methods are rapid, reproducible, highly sensitive and applicable to the determination of the agent in plasma, urine and cerebrospinal fluid.



Bisantrene .
NSC-337766

Figure 1. Structure of 9,10-anthracenedicarboxaldehyde bis[4,5-dihydro-1H-imidazole-2-yl) hydrazone] dihydrochloride.

MATERIALS AND METHODS

Chemicals

Bisantrene (NSC-337766) was kindly supplied by Lederle Laboratories Division, American Cyanamid Company, Pearl River, NY. Glass distilled methanol was obtained from Burdick and Jackson labs (Saginaw, MI, USA). All other chemicals were obtained from regular commercial suppliers.

Sample Preparation

Biological samples were obtained from patients receiving 40-250 mg/m^2 of Bisantrene intravenously. Blood was drawn into heparinized tubes and then centrifuged at 12,000 X g for 10 min. in a Sorvall RC2-B centrifuge to separate plasma from red blood cells. Urine was collected as voided. A C_{18} Sep-Pak (Waters Associate, Milford, MA) was used as a minichromatographic column to prepare the biological samples (3). The cartridge was first eluted with 4 ml of methanol, followed by 4 ml of equal volumes of methanol and water, 10 ml of 0.05 M sodium phosphate, 3 ml of biological samples, and then 4 ml of 0.05 M sodium phosphate. The above eluates were discarded. The column was washed with 6 ml of chloroform:methanol (2:1,v/v), and the eluent was collected in a drying cup and evaporated to dryness under a stream of nitrogen in a Brinkmann model SC/48 evaporator (Brinkmann

Instrument Co., Westbury, NY). It was then reconstituted with 50 µl of N-N-dimethyl acetamide and 250 µl of saline for the high performance liquid chromatography (HPLC) separation. Those urine samples that remained cloudy after reconstitution were recentrifuged at 12,000 X g for 15 min. and the supernatent collected again for injection into the HPLC.

HPLC Analysis

All analyses were performed on a Waters Associates liquid chromatograph (Milford, MA, USA) equipped with a Model M-6000A pump, U6K injector, and Schoeffel Model SF-970 fluorescent detector, (Kratos Analytical Company, Westwood, NJ) with the excitation wavelength set at 260 nm and emission set at 550 nm. Separation was achieved on an analytical reverse-phase µ Bondapak C_{18} column (Waters Associates, 4 mm x 30 cm, 10-mm particle size) using 0.02 M Ammonium acetate in 40% methanol, pH 4.0, as eluant at a flow rate of 1.5 ml/min. Retention times were determined by a Shimadzna chromatopec-EIA electronic integrator (Kyoto, Japan) and Brinkmann Instrument Model 2544 recorder.

Results and Discussion

Using the conditions described above, Bisantrene was eluted 8.5 min. after injection. In the urine samples, the separation of Bisantrene from other components may have been delayed by 0.2 to 0.5 min due to the presence of normal urinary constituents (Fig. 2). The preparation of the drug using C_{18} Sep-paks along with the procedures of evaporation, centrifugation and transfer of samples resulted in 75% recovery of the drug.

The standard curves for Bisantrene in plasma and in urine are shown in Fig. 3. The concentration is linear over a range of 125 to 2000 ng/ml. The lower limit of detection was approximately 2 ng/ml.

Figure 4 is a chromatograph of a patient's plasma after Bisantrene was administered at a dose of 250 mg/m^2. This HPLC technique is rapid, sensitive and reproducible. It provides easy detection of Bisantrene in both plasma and urine of patients receiving the drug. This assay is

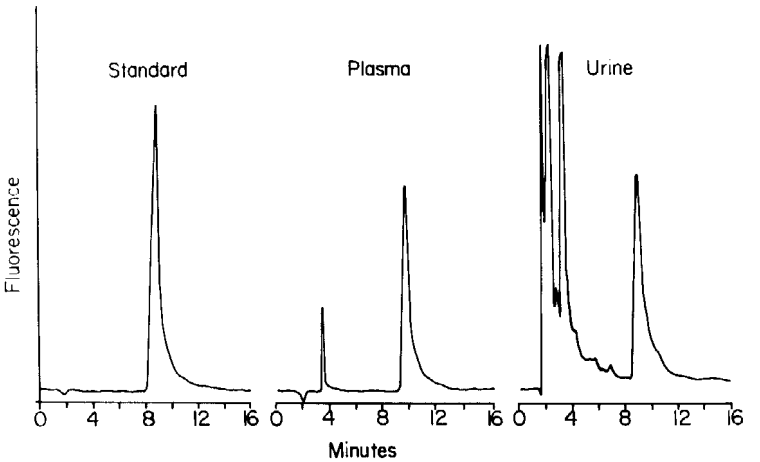


Figure 2. Elution profile of standard Bisantrene in aqueous solution (left), in human plasma (center) and in human urine (right) with fluorescence at 550 nm.

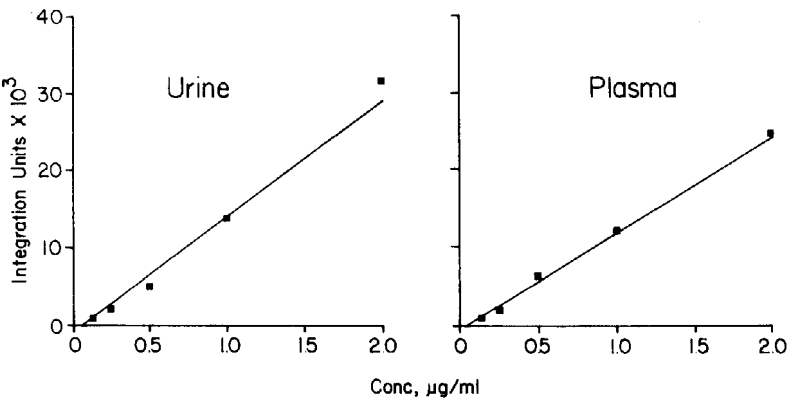


Figure 3. Standard curves of Bisantrene in plasma and urine.

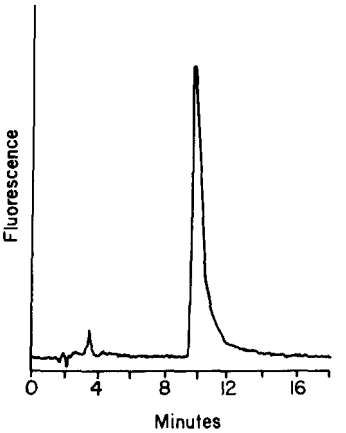


Figure 4. Chromatogram of a patient's plasma immediately after receiving 250 mg/m² of Bisantrene.

currently being used to study the clinical pharmacology of Bisantrene at our institution.

ACKNOWLEDGEMENT

Supported by Contract NO1-CM-87185 from the Division of Cancer Treatment, National Cancer Institute. We wish to thank the American Cyanamid Company for providing us with this drug.

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