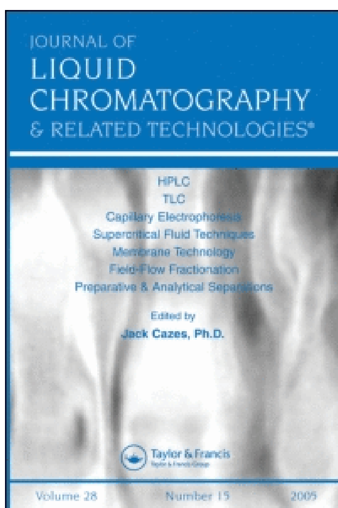
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



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

Analysis of the Antitumor Agent Bay i 7433 (Copovithane) in Plasma and Urine by High Performance Liquid Chromatography

Michael G. Rosenblum[a]; Gabriel N. Hortobagyi[b]; W. Wingender[c]; Evan M. Hersh[a]
[a] Clinical Immunology and Biological Therapy, The University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, TX [b] Internal Medicine, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX [c] Bayer AG, Institute for Pharmacokinetics, Wuppertal, Germany

To cite this Article Rosenblum, Michael G. , Hortobagyi, Gabriel N. , Wingender, W. and Hersh, Evan M.(1984) 'Analysis of the Antitumor Agent Bay i 7433 (Copovithane) in Plasma and Urine by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 7: 1, 159 — 166

To link to this Article: DOI: 10.1080/01483918408073956

URL: http://dx.doi.org/10.1080/01483918408073956

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(1), 159-166 (1984)

ANALYSIS OF THE ANTITUMOR AGENT BAY i 7433 (COPOVITHANE) IN PLASMA AND URINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Michael G. Rosenblum[1], Gabriel N. Hortobagyi[2], W. Wingender[3], and Evan M. Hersh[1]

[1]Clinical Immunology and Biological Therapy; [2]Internal Medicine, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030. [3]Bayer AG, Institute for Pharmacokinetics, Wuppertal, Germany

Requests for reprints should be addressed to:
Rosenblum, Ph.D. Department of Clinical Immunology
and Biological Therapy M.D. Anderson Hospital and
Tumor Institute Houston, Texas 77030.

Downloaded At: 17:22 24 January 2011

ABSTRACT

Copovithane (BAYi7433) is a synthetic polymer of molecular weight 7,800 daltons with antitumor activity. An analytical method for copovithane in biological fluids involving organic extraction, and hydrolysis and TNBS derivitization of generated methylamine was developed. This method utilizes HPLC for final quantitation of the TNBS-methylamine adduct. The lower limit of detection was 15 g/ml of either plasma or urine. This method was sensitive enough to monitor the pharmacokinetics of copovithane in patients receiving therapy.

INTRODUCTION

Bay i 7433 (copovithane, Fig. 1) is a synthetic copolymer of 1, 3 bis-(methylaminocarboxy)-2-methylene propane and N-vinylpyrrolidone with an average molecular weight of 5,800 daltons. Although uncharged, this high molecular weight material is highly soluble in aqueous solvents. Copovithane was found to have significant antitumor activity against sarcoma 180, P388 leukemia, carcinoma E0771 and fibrosarcoma F1026 in mice and activity against Walker 1098 tumor in rats. Copovithane was found to be a well-tolerated compound which exhibited antitumor activity in animal studies after single injection either prior to or after innoculation of tumor cells (1).

Copyright © 1984 by Marcel Dekker, Inc. 0148-3919/84/0701-0159$3.50/0

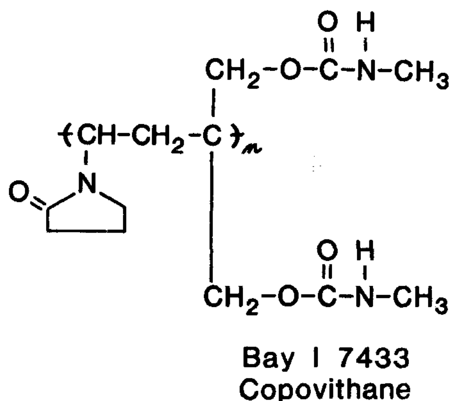


FIGURE 1: Subunit structure of copovithane polymer.

Downloaded At: 17:22 24 January 2011

The mechanism of antitumor action of this agent has not yet been elucidated. However, copovithane is not cytotoxic in vitro, suggesting that this agent requires metabolic conversion or that this agent operates through changes in host control mechanisms (1). Copovithane is currently undergoing phase I clinical trials and in concert with these trials, we have developed a high performance liquid chromatographic method to assay copovithane in biological fluids in preparation for pharmacokinetic studies in man.

MATERIALS AND METHODS

All materials purchased from regular commercial suppliers were of reagent grade or higher. Distilled-in-glass acetonitrile was purchased from Burdick and Jackson, Muskegon, Michigan. All solvents were filtered, vacuum degassed and sparged with nitrogen immediately before use.

Chromatography. All analyses were performed with a Waters Associates (Milford, MA) liquid chromatograph consisting of a model 710B sample processor, a model M6000 pump, a model 720 system controller, a data module and a model 450 variable-wavelength UV detector. An analytical reverse phase (30 cm x 3.9 mm, 10 μ particle size) C-18 column from Waters Associates was used for all analyses. The mobile phase consisted of 30% acetonitrite and 70% water. The

flow rate was 2 ml/min. The column eluate was monitored for UV absorbance at 340 nm.

Plasma. Aliquots (2 ml) of plasma containing copovithane were placed in 15 ml Corex test tubes and cooled on ice for 5 minutes. Plasma proteins were precipitated by the addition of 200 μl of 10 N perchloric acid. The samples were vortexed vigorously and allowed to stand for 5 minutes on ice. The samples were spun at 17,000 x g for 15 minutes, the supernatents were transferred to glass (12 x 75 mm) test tubes and 200 μl of 10 N KOH was added to neutralize each sample. The samples were again cooled on ice for 5 minutes and then centrifuged for 3 minutes in a Serofuge II centrifuge. The supernatents were transferred to 15 ml Corex test tubes and 2 ml of hot (85°C) saturated sodium chloride solution was added to each sample. The drug was then extracted three times by addition of 3 ml of chloroform (Fisher Science Co., Fairlawn, New Jersey). The samples were vortexed vigorously and then spun at 17,000 x g for 15 minutes. The chloroform extracts were combined and then dried down under a nitrogen stream. The samples were then reconstituted with 1 ml of 5 N hydrochloric acid, quantitatively transferred to a 3.5ml screw-cap vial, sealed and placed in a 160°C oil bath. After 16 hours, the samples were removed, cooled on ice, and adjusted to neutrality with 1 ml of 5 N sodium hydroxide. The pH of each sample was further adjusted to 8.0 by the addition 3 ml of 1 M sodium bicarbonate buffer, pH 8.0. An aliquot (0.75 ml) of a 0.5% solution of trinitrobenzene sulphonic acid (TNBS, Sigma Chemical Co., MO) in acetone was added. The samples were then incubated in the dark for 150 min. and were extracted 3 times with 3 ml of ethyl acetate. The ethyl acetate extracts were combined and evaporated to dryness under a nitrogen stream. The samples were reconstituted in 250 μlof 0.2 M Na_2HPO_4 (pH 6.4) in acetonitrile and chromatographed as described.

RESULTS AND DISCUSSION

Copovithane (BAY i 7433) is a poly-n-methyl carbamate polymer. Since this molecule has little intrinsic UV character, chemical modification of its structure to incorporate a chromophore was necessary to increase sensitivity in the drug assay.

Downloaded At: 17:22 24 January 2011

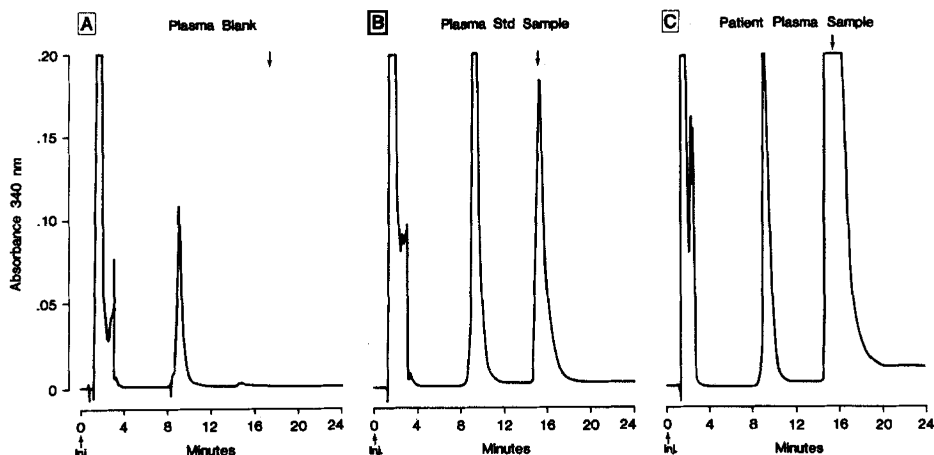


FIGURE 2: High performance liquid chromatography of copovithane.

2A: HPLC of plasma blank. This sample corresponds to injection of 2 ml of plasma.

2B: HPLC of plasma with added copovithane (40 μg/ml).

2C: HPLC of patient plasma sample after administration of copovithane. Quantitation of copovithane in this ample is approximately 150 μg/ml.

Downloaded At: 17:22 24 January 2011

Figure 2A shows the high performance liquid chromatogram of a plasma blank. The arrow at 15 minutes indictes the position of the TNBS methylamine adduct derived from copovithane hydrolysis. At this figure shows, the plasma blank contains no peaks which interfere with chromatographic analysis. Figure 2B shows a plasma sample with copovithane added at a concentration of 400 μg/ml. Figure 2C shows a patient plasma sample obtained 1 hour after the administration of copovithane at a dose of $10g/m^2$. Copovithane concentration in this sample was calculated to be 400 μg/ml. A standard curve for copovithane in plasma was constructed by the method of standard addition. The results are shown in Figure 3. The constructed curve closely fit (r^2 = 0.995) the calculated line, y = 1.22 x -10.2 over the concentration range 0 to 500 μg/ml. The calculated recovery rate using this method for plasma was 40%. Interassay and intra-assay variability of standards was 19% and 2.3% respectively. The functional lower limit of detection of this method in plasma was 15 g/ml.

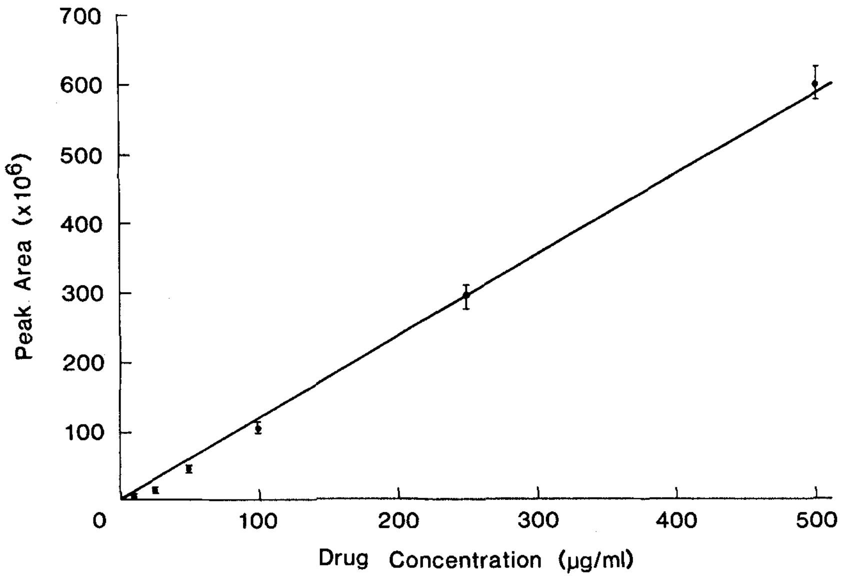


FIGURE 3: Standard curve for copovithane in plasma.

Downloaded At: 17:22 24 January 2011

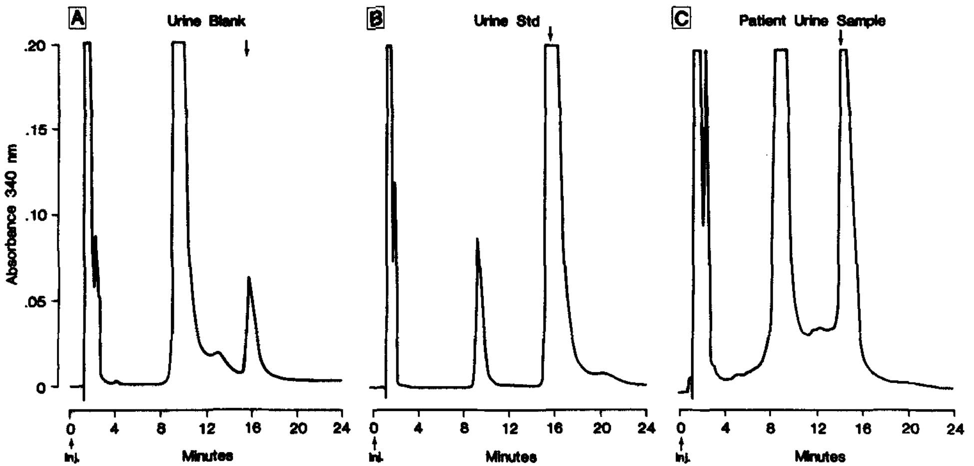


FIGURE 4: High performance liquid chromatography of copovithane in urine.

4A: HPLC of urine blank from a normal volunteer.

4B: HPLC of urine with added copovithane (100 μg/ml).

4C: HPLC of patient urine sample twenty-four hours after drug administration (75 μg/ml).

Incubation of copovithane with plasma at 37°C for 24 hours showed no decrease in drug concentration compared to a freshly prepared sample.

Figure 4A shows the HPLC profile of a urine blank. At approximately 16 min, figure 4A shows the presence of an endogenous peak which interferes with copovithane quantitation at low drug concentration. Attempts to further reduce this interference were unsuccessful.

Figure 4B shows a urine standard with added copovithane at a concentration of 100 μg/ml. The calculated recovery rate using this method was 80% for urine. Figure 4C shows the chromatogram of a urine sample collected from a patient in the first 24 hours after copovithane administration.

For quantitative purposes, a standard curve for urine was constructed also by this method of standard addition. As shown in figure 5, this assay was linear over the entire concentration range tested. Linear regression analysis showed

Downloaded At: 17:22 24 January 2011

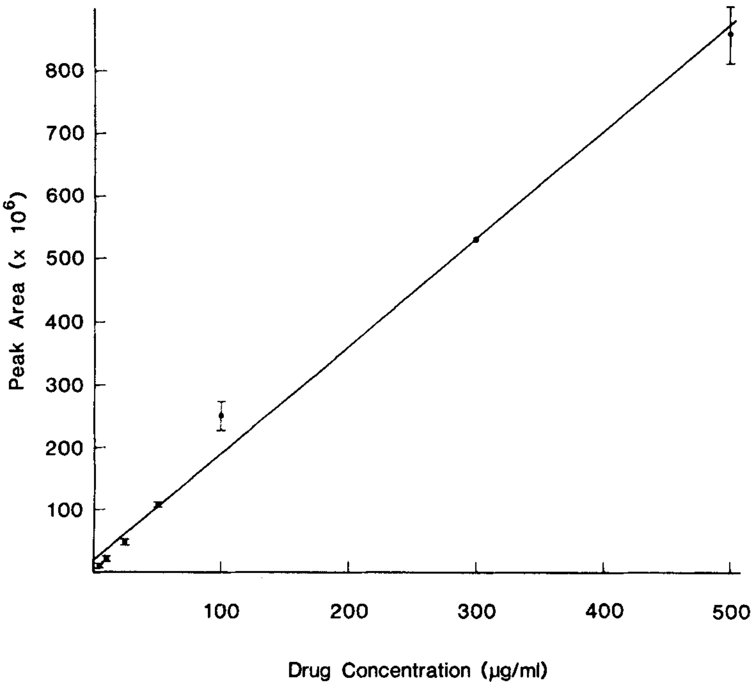


FIGURE 5: Standard curve for copovithane in urine.

that the points closely fit (r^2 = 0.95) a straight line. The lowest limit of detection using this method was approximately 15 μg/ml.

Preliminary pharmacokinetic analysis of plasma obtained from one patient who received copovithane as a 20 minute infusion at a dose of 10 g/m^2 is shown in figure 6. Assay sensitivity limits for plasma were reached after 12 hours. In this one patient, $t_{1/2}$-α was 18 minutes while the terminal phase $t_{1/2}$ was 180 minutes.

Complete pharmacokinetic analysis of copovithane administration will be presented upon completion of this clinical trial. However, this study shows that the method is sensitive enough to monitor plasma concentrations of copovithane at pharmacologically achievable doses.

Downloaded At: 17:22 24 January 2011

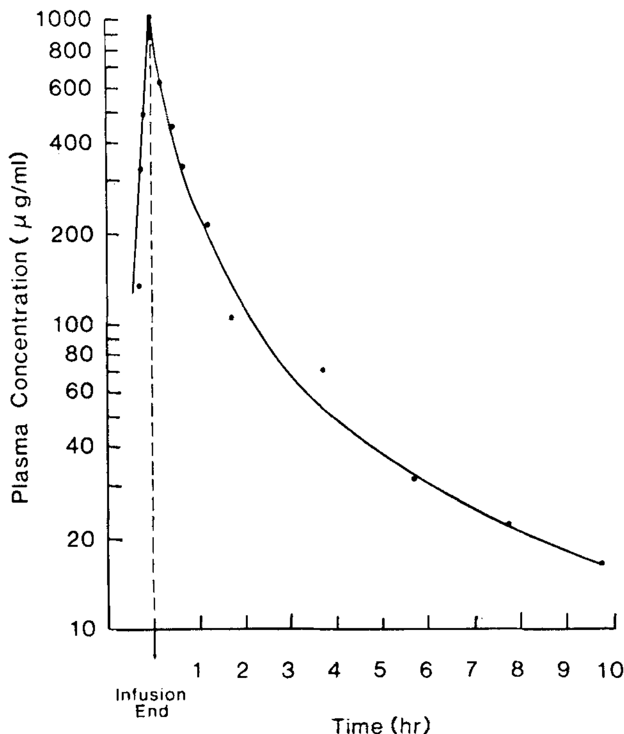


FIGURE 6: Plasma disappearance of copovithane in one patient who received 10 g/m^2 dose as a one-hour infusion.

REFERENCES

1. Schlumberger HD: Bayi7433, a synthetic polymer with antitumor activity. In: Augmenting Agents in Cancer Therapy - Current Status and Future Prospects. E. Hersh and M. Chirigos (eds). Raven Press, 1980.

Downloaded At: 17:22 24 January 2011