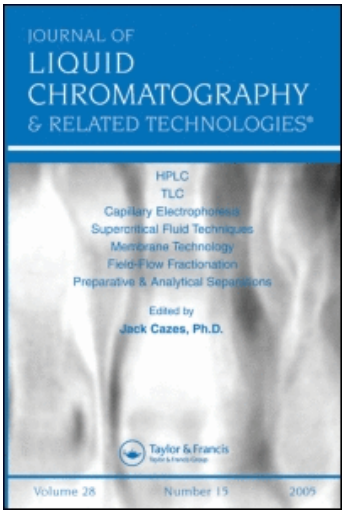

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DETERMINATION OF ADRIAMYCIN AND ACLACINOMYCIN A IN PLASMA BY HIGH PRESSURE LIQUID CHROMATOGRAPHY AND SPECTROPHOTOFLUOROMETRY

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ABSTRACT

Procedures for the determination of adriamycin and aclacinomycin A in plasma using high pressure liquid chromatography for resolution and fluorescence for detection are described. Separations of these anthracyclines from some of their metabolites--adriamycinol and 4'''-hydroxy aclacinomycin A isomers--are also illustrated.

INTRODUCTION

Adriamycin (ADR) is representative of a number of Streptomyces-produced anthracycline antibiotics that are effective anticancer agents (1,2). Several high pressure liquid chromatographic (LC) procedures have been developed to study the pharmacokinetics of anthracyclines. ADR was resolved from its major mammalian metabolite, adriamycinol (AMNOL), by a gradient elution technique on diphenyl- and cyanoamino polar bonded supports (3,4,5) and it has been employed as an internal standard in a procedure that resolved daunomycin and daunomycinol on silica microspheres (6). Others (7) concluded that silica was the most efficient support for the analy-

sis of pharmaceutical preparations of ADR that contained adriamycinone and other impurities.

Because ADR exhibits bone marrow suppression and cumulative dose-dependent cardiotoxicity (8,9), other anthracycline derivatives have been developed and are undergoing preliminary clinical trials. These include aclacinomycin A (ACLA) and some of its isomers (10).

In the present communication, we describe two procedures that combine efficient silica columns and sensitive fluorescence monitoring to determine ADR, AMNOL, and ACLA. In addition, the method presented for ACLA is shown to be applicable to the separation of ACLA and its two 4'''-hydroxy isomers.

EXPERIMENTAL

Drugs and Reagents

Reference samples (5 mg) of ADR and AMNOL were obtained from Drs. W. W. Lee and T. H. Smith of the Bioorganic Chemistry Department, SRI International. Dr. T. Oki (Sanraku-Ocean Co., Ltd., Tokyo, Japan) provided analytical samples of ACLA and its 4'''-hydroxy isomers, MA-144-N1 (terminal sugar, L-rhodinose) and MA-144-M1 (terminal sugar, L-amicetose), respectively. Structural formula of the parent compounds are shown in Figure 1.

Chloroform, methanol, and ethyl acetate were "distilled in glass" reagents (Burdick and Jackson, Muskegon, MI), dimethyl sulfoxide was of spectroquality (Matheson, Coleman, and Bell, Norwood, OH), and water was glass-distilled after deionization. Ammonium hydroxide (28% w/v), ethylene glycol, and all other materials were reagent grade chemicals.

Chromatography

Separations were performed at ambient temperature (22°C) on a prepacked, 5-μm LiChrosorb SI 60 column (3.2 x 250 mm, Altex Scientific Co., Berkeley, CA) using a mobile phase of chloroform-methanol-ammonium hydroxide-water (855:130:10:5) for ADR and AMNOL and one

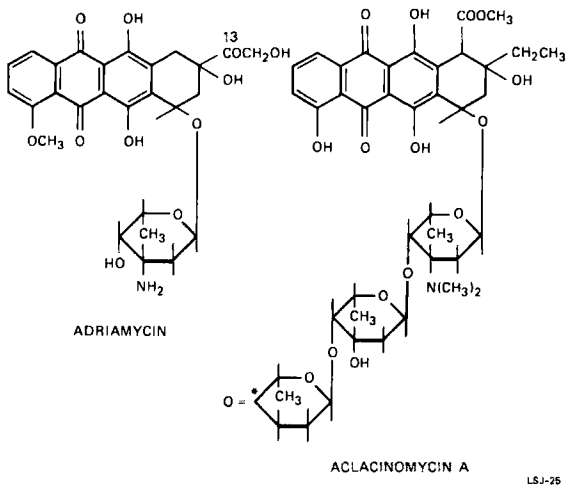

FIGURE 1

Structures of adriamycin (ADR) and aclacinomycin A (ACLA). The asterisk in the formula of ACLA indicates the 4'''-position

consisting of chloroform-dimethyl sulfoxide-ethylene glycol (975:20:5) for ACLA, MA-144-M1, and MA-144-N1. In both cases, the mobile phase flow rate was 1.1 ml/min and injections of aliquots (100 µl) onto the columns were made using a Model 70-10 loop injector (Rheodyne, Berkeley, CA).

Fluorescence of the effluents from the columns was measured as it flowed through a 200-µl quartz cell (Markson Science, Inc., Delmar, CA) in a modified Aminco-Bowman spectrophotofluorometer that we have previously described (11). ADR and AMNOL were detected by their fluorescence at 580 nm (excitation, 475 nm); ACLA, by its fluorescence at 520 nm (excitation, 460 nm). Ultraviolet monitoring of ACLA, MA-144-M1, and MA-144-N1 was performed at 254 nm using an Altex Model 153 detector equipped with an 8-µl flowcell.

Extractions

ADR and AMNOL were extracted from 0.40 ml of rabbit plasma (buffered by 0.10 ml of 1 M phosphate buffer, pH 8.0) by addition

of 2.0 ml of chloroform-methanol (9:1) followed by gentle mechanical shaking for 20 min. After centrifugation to separate the phases, the aqueous layer was discarded and 1.6 ml of the organic phase was evaporated to dryness at 20°C under a stream of high purity N_2. The residue was dissolved in 150 μl of the mobile phase and a 100-μl portion was chromatographed. ACLA was extracted under similar conditions except that the plasma was buffered by addition of 0.10 ml of 1 M phosphate, pH 7.0 and the extraction was performed with ethyl acetate.

RESULTS AND DISCUSSION

Figure 2A shows a typical fluorescence elution profile of extracts of plasma from a female New Zealand white rabbit before commencing and after cessation of a 2-hr intravenous infusion of 20 mg ADR·HCL/kg. ADR and AMNOL were completely resolved from each other, exhibiting retention times of 2.8 and 6.2 min, and plasma concentrations of 243 and 97 ng/ml, respectively. ADR was not fully resolved from several smaller peaks that appeared in treatment plasma but extractions of ADR standards from control plasma did not exhibit these smaller LC peaks. Perhaps the use of a smaller flow cell would have achieved better resolution. Such a flow cell (20-μl) is now available from Precision Cell, Inc. (Hicksville, NY) and is designed for use in a standard fluorometer cell holder (12.5 × 12.5 mm). Comparison of the pretreatment and treatment plasmas clearly indicated that plasma alone did not contribute fluorescence.

Figure 3 shows the fluorescence response of ADR (solid circles) extracted from rabbit plasma over a concentration range of 0.00187 to 0.937 μg ADR/ml (0.002 to 1.0 μg ADR·HCl/ml) of plasma. Clearly, an excellent linear regression ($r = 0.9999$, $P < 0.001$) was obtained. In a separate experiment, we determined the recovery of ADR from rabbit plasma by comparing the fluorescence re-

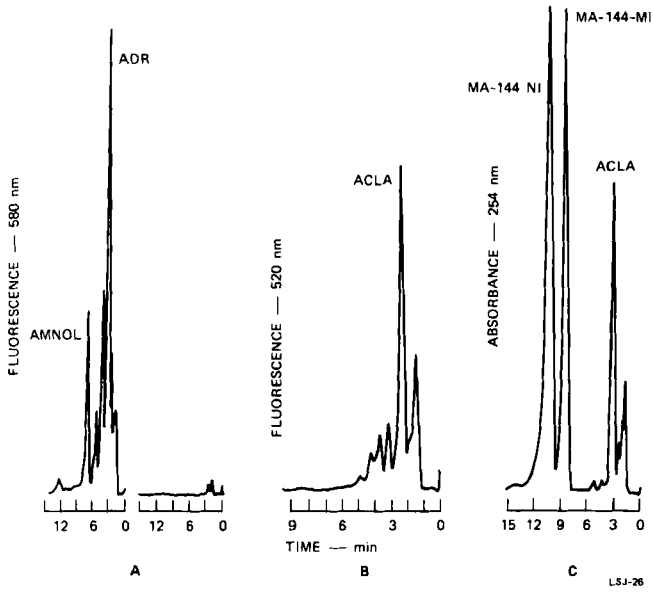

FIGURE 2

A. Elution profile of ADR and AMNOL extracted from rabbit plasma after ADR treatment (left) and of an extract of pretreatment plasma (right).

B. Elution profile of ACLA extracted from rabbit plasma.

C. Elution profile of ACLA, MA-144-M1, and MA-144-N1 injected onto the column.

sponse of extracted samples with that obtained by direct injections of ADR·HCl. The results showed a quantitative recovery over a range from 0.00937 to 0.937 μg ADR/ml (0.010 to 1.0 μg ADR·HCl/ml) of plasma. Without applying a correction for phase volume changes, the mean recovery ± the standard error of ADR was 116 ± 0.2%. The recovery range was exceedingly narrow and reproducibility of a limited number of individual tests was within ± 4%. Fluorescence noise was equivalent to approximately 1 ng of ADR/ml of plasma and the lower limit of practical sensitivity was 2 to 4 ng ADR/ml.

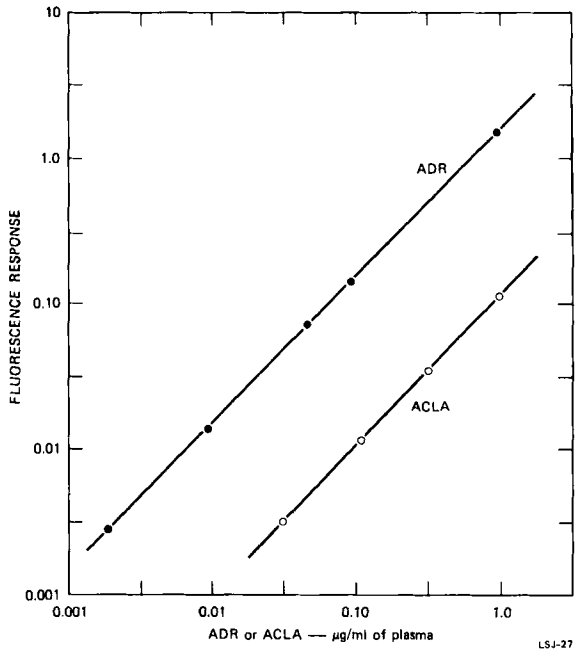

FIGURE 3

Fluorescence response of ADR (●) and ACLA (o) as a function of plasma concentration. Peak heights were used for quantitation.

Figure 2B shows the fluorescence elution profile of extracts of control rabbit plasma that contained 1.0 µg ACLA/ml. The retention time of ACLA was 2.6 min at a flow rate of 1.1 ml/min. In this case, plasma alone contributed more interference than that found in the procedure used for ADR. The increased interference was probably due to a greater extraction of endogenous material by ethyl acetate, which was used to increase the extraction efficiency for ACLA.

Figure 3 also shows the fluorescence response of ACLA (open circles) extracted from rabbit plasma over a concentration range of 0.05 to 1.0 µg ACLA/ml of plasma. Clearly, an excellent linear regression ($r = 0.9993$, $P < 0.001$) was obtained. Also, we determined the recovery of ACLA from rabbit plasma by comparing the fluorescence

response of extracted samples with that obtained by direct injections of ACLA onto the column. The results showed that the mean recovery ± the standard error was 78.6 ± 2.9% over a range from 0.05 to 1.0 μg ACLA/ml of plasma. Reproducibility of a limited number of duplicate tests was within ± 2% of their mean. Fluorescence noise was equivalent to approximately 10 ng ACLA/ml of plasma and the lower limit of practical sensitivity was 10 to 20 ng ACLA/ml.

Recently, Oki (12) showed that ACLA is reduced metabolically in vitro by mammalian tissue homogenates to the 4''' hydroxy isomers of ACLA. To determine the applicability of our LC system to separate these reduced isomers of ACLA, we chromatographed small amounts of the isomers in the presence and absence of ACLA. The eluted compounds were detected at 254 nm and the retention times for ACLA, MA-144-M1, and MA-144-N1, were 3.1, 8.5, and 10.1 min, respectively, at a flow rate of 1.0 ml/min. Figure 2C shows the resolution of the two isomers from each other and from ACLA.

Because fluorescence provides a greater potential for sensitive detection than ultraviolet absorption, we determined the fluorescence characteristics of the 4''' -hydroxy derivatives of ACLA in the mobile phase used for ACLA. The activation and fluorescence maxima and fluorescence intensity relative to ACLA were 470 nm, 545 nm, and 100% and 470 nm, 540 nm, and 127%, for MA-144-M1 and MA-144-N1, respectively. These results suggest that ACLA and the 4''' -hydroxy isomers could be quantitated by fluorescence after LC. No studies on the extraction of the 4''' -hydroxy isomers from plasma were performed, however.

ACKNOWLEDGEMENT

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REFERENCES

1. Blum, R. H. and Carter, S. K., Adriamycin--a new anticancer drug with significant clinical activity. Ann. Intern. Med., 80, 249, 1974.

2. Carter, S. K., Adriamycin--a review. J. Natl. Cancer Inst., 55, 1265, 1975.

3. Langone, J. J., Van Vunakis, H., and Bachur, N. R., Adriamycin and metabolites: Separation by high-pressure liquid chromatography and quantitation by radioimmunoassay. Biochem. Med., 12, 283, 1975.

4. Israel, M., Pegg, W. J., Wilkinson, P. M., and Garnick, M. B., HPLC applications in the analysis of adriamycin and analogs in biological fluids. In Biological/Biomedical Applications of Liquid Chromatography, Hawk, G. L., editor, Marcel Dekker, Inc., New York, in press, 1978.

5. Israel, M., Pegg, W. J., and Wilkinson, P. M., Urinary anthracycline metabolites from mice treated with adriamycin and N-trifluoroacetyladriamycin-14-valerate. J. Pharmacol. Exp. Therap., 204, 696, 1978.

6. Hulhoven, H. and Desager, J. P., Quantitative determination of low levels of daunomycin and daunomycinol in plasma by high-performance liquid chromatography. J. Chromatog., 125, 369, 1976.

7. Barth, H. G. and Conner, A. Z., Determination of doxorubicin hydrochloride in pharmaceutical preparations using high pressure liquid chromatography. J. Chromatog., 131, 375, 1977.

8. Lefrak, E. A., Pivha, J., Rosenheim, S., and Gottlieb, J. A., A clinicopathologic analysis of adriamycin cardiotoxicity. Cancer, 32, 302, 1973.

9. Mettler, F. P., Young, D. M., and Ward, J. M., Adriamycin-induced cardiotoxicity (cardiomyopathy and congestive heart failure). Cancer Res., 37, 2705, 1977.

10. Oki, T., Matsuzawa, Y., Yoshimoto, A., Numata, K., Kitamura, I., Hori, S., Takamatsu, A., Umezawa, H., Ishizuka, M., Naganawa, H., Suda, H., Hamada, M., and Takeuchi, T., New antitumor antibiotics, aclacinomycins A and B. J. Antibiotics, 18, 830, 1975.

11. Murray, J. F., Jr., Gordon, G. R., Gulledge, C. C., and Peters, J. H., Chromatographic-fluorometric analysis of antileprotic sulfones. J. Chromatog., 107, 67, 1975.

12. Oki, T., New anthracycline antibiotics. Japan. J. Antibiotics, 30 (Supplement), S-70, 1977.