Waters
Lab Highlights
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DETERMINATION OF ADRIAMYCIN, ADRIAMYCINOL, AND THEIR 7-DEOXYAGLYCONES IN HUMAN SERUM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Adriamycin is a natural occurring anthracycline glycosidic antibiotic with a broad spectrum of antitumor activity in human cancer. Cummings et al. (1) describe an 18 minute reversed-phase isocratic high performance liquid chromatographic assay for the measurement of adriamycin, and its major glycoside metabolite, adriamycinol, as well as four different aglycone metabolites (adriamycinol aglycone, adriamycinol 7-deoxyaglycone, adriamycin aglycone, and adriamycinol 7-deoxyaglycone) and daunorubicin (internal standard) in human serum. The lower limit of detection in serum is 3 ng/ml for adriamycin and I ng/ml for adreamycinol and the 7-deoxyaglycones with coefficients of variation for k' of less than 5% throughout the day. The extraction technique for serum is capable of an almost equal recovery (<77%) of adriamycin, metabolites and daunorubicin without interference from endogenous serum components.

Figure 1 shows the separation of a mixture of standards. Figure 2A shows a serum blank and Figure 2B shows the serum extract from a patient who reeceived adriamycin as an intravenous bolus of 40mg/m^2.

Figure 1

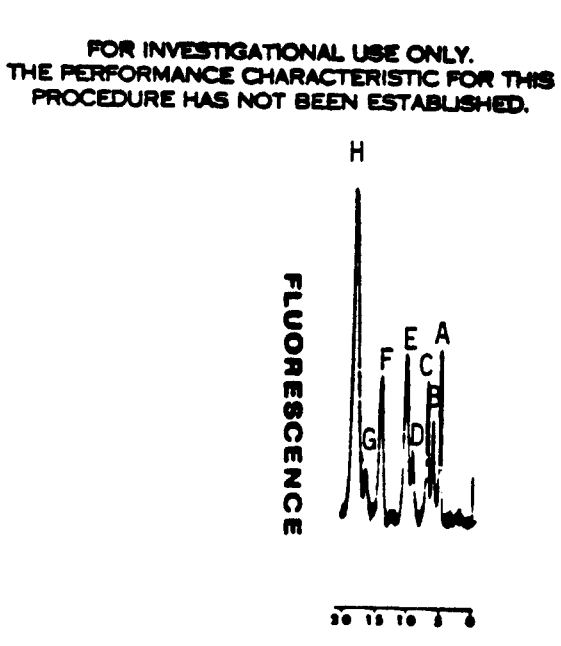
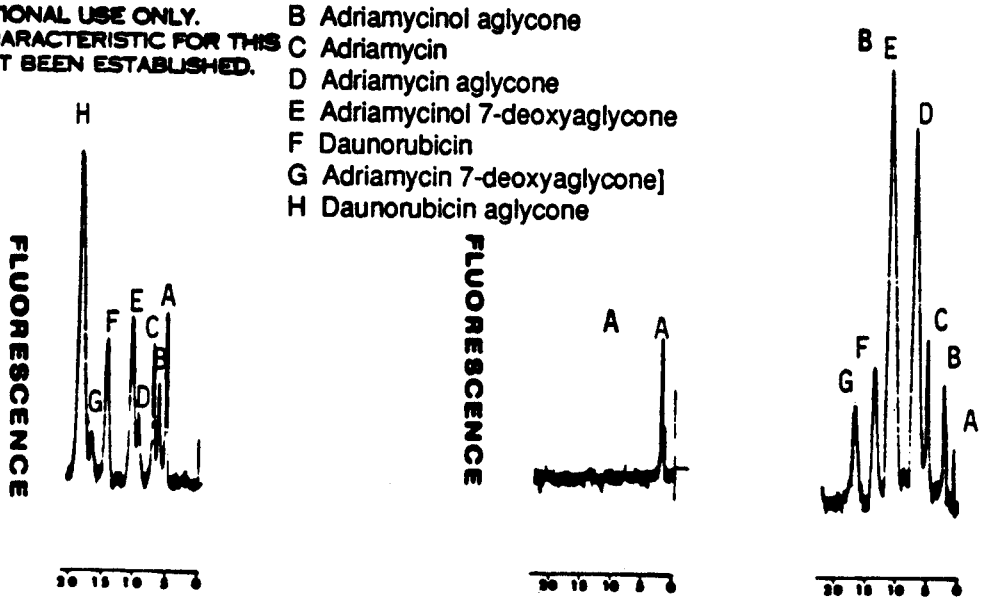


Figure 2



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Chromatographic Conditions:

Column:	μBondapak™ C18 packing material (4.6mm X 25cm)
Mobile Phase	Water: Methanol: Acetonitrile: Propan-2-ol (62.5:12.5:12.5:12.5) with 5mM orthophosphoric acid
Flow Rate:	2.5 ml/min
Detection:	Fluorescence 480 nm (ex) 560 nm (em)

Extraction Conditions:

1. Collect blood sample from patient in plain glass tubes (heparinized tubes were not used because heparin interfered with HPLC assay) and allow to clot for 1 hour at 4°C.

2. Centrifuge at 1000 g for 10 minutes, remove the serum and store at -20°C till anslysis time.

3. Thaw serum, to 1 or 2 ml of serum, add 10μl of methanol containing daunorubicin (100 ng), add 5 or 10 ml of chloroform: propan-2-0l (2:1) and vortex.

4. Centrifuge at 2000 g for 15 minutes at 4°C.

5. Transfer bottom layer to clean test tube, evaporate to dryness, reconstitute in 50 or 100μl of methanol and inject 20μl.