taining the drugs did not require concentration. The coefficients of variation for the standard curves at the low and high concentrations were 3.89 and <1%, respectively. The analysis of acecainide in rat feed is linear, precise, and accurate over the needed concentration range. The construction of a standard curve spanning the desired concentration range on each day of analysis assures good quantitation of drug in the feed.

Procainamide hydrochloride was analyzed from 0.50 to 50.0 mg/g of feed. Standards were determined in quadruplicate at 0.5, 1.0, 2.0, and 5.0 mg/g of feed. Over this concentration range, the linear regression exhibited a correlation coefficient of 0.9997, an intercept of −0.067, and a slope of 0.872. Increasing the concentration of the internal standard again allowed extension of the analytical range. Standards determined in quadruplicate at 5.0, 10.0, 20.0, and 50.0 mg/g of feed exhibited a correlation coefficient of 0.9998, an intercept of 0.027, and a slope of 0.063. The coefficient of variation for both standard curves was <1%. The assay of procainamide hydrochloride from rat feed is linear, precise, and accurate over the desired concentration range.

Acecainide hydrochloride in rat feed was tested and shown to be stable (85–115% of the theoretical amount) for up to 5 months at ambient temperature. Procainamide hydrochloride exhibited some instability over the same period, but the cause or result of this instability was not determined.

Acecainide and procainamide may be mixed with rat feed and accurately analyzed with this procedure. The drugs were stable in rat feed if kept at or below room temperature and utilized within a few months.

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Analysis of 4′-Demethylepipodophyllotoxin-9-(4,6-*O*-ethylidene-β-D-glucopyranoside) by High-Pressure Liquid Chromatography

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Abstract □ A rapid and specific high-pressure liquid chromatographic assay is described for the quantitative analysis of 4′-demethylepipodophyllotoxin-9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (I) in plasma. After chloroform extraction, I was analyzed by reversed-phase chromatography and UV detection (252 nm). The maximum sensitivity was 0.1 μg/ml. Quantitation was by relative response factor calibration using an integrating microcomputer. Over the concentration range of 0.5–90 μg/ml, the average recovery of I from plasma was 95.4 ± 3.8% (*SD*).

Keyphrases □ 4-Demethylepipodophyllotoxin -9- (4,6-*O*-ethylidene-β-D-glucopyranoside)—high pressure liquid chromatographic analysis □ High-pressure liquid chromatography—analysis, 4-demethylepipodophyllotoxin-9-(4,6-*O*-ethylidene-β-D-glucopyranoside) □ Antitumor activity—4-demethylepipodophyllotoxin -9- (4,6 -*O*- ethylidene-β-D-glucopyranoside), high-pressure liquid chromatographic analysis

The semisynthetic epipodophyllotoxin analog 4′-demethylepipodophyllotoxin-9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (I) has shown activity against a variety of human tumors (1). Since I is undergoing advanced clinical investigation, it is desirable to monitor plasma drug concentrations from different dosage regimens and from drug interaction studies. Analysis of I previously was carried out by radioisotopic techniques (2–4). This report discusses a high-pressure liquid chromatographic (HPLC) method developed for the quantitation of I in plasma.

EXPERIMENTAL

Apparatus—The analyses of I were conducted using a liquid chromatograph[1] operated at ambient temperature and equipped with a variable-wavelength detector (252 nm). Separations were performed on a 250 × 3.0-mm i.d. reversed-phase column[2]. Samples were introduced onto the column through a septumless injector (10 μl) coupled to an automatic sampler injection system[1]. The chromatograms were traced on a strip-chart recorder with the peak area integration performed by a microcomputing data system[1].

Reagents and Solvents—Compound I was obtained as a gift[3]. All chemical reagents were analytical grade except for methanol and dioxane, which were pesticide residue or HPLC quality[4].

Chromatographic Conditions—The mobile phase was a 50:50 mixture of 5 m*M* KH_2PO_4–NaOH buffer (pH 7.8) and methanol with the flow rate adjusted to 40 ml/hr (~1000 psi). A precolumn[2] was used to minimize the effect of extraneous plasma-extracted material on the performance of the reversed-phase column.

Extraction Procedure—Aliquots (2.0 ml) of plasma were transferred to 15-ml thick-wall centrifuge tubes[5], diluted with 2 ml of water, and extracted with 4 ml of chloroform. The mixture was vortexed vigorously for 30 sec, and the phases were separated by centrifugation (15 min at 10,000×*g*). The lower organic layer was removed by a Pasteur pipet (24

[1] Model 8500, Varian Co., Sunnyvale, Calif.
[2] Partisil-10 ODS, Whatman Inc., Clifton, N.J.
[3] Also known as VP-16-213 and NSC-141540; supplied by Ms. Kozkuz, Sandoz Pharmaceuticals, Hanover, N.J.
[4] Burdick & Jackson Laboratories, Muskegon, Mich.
[5] Corex No. 8441.

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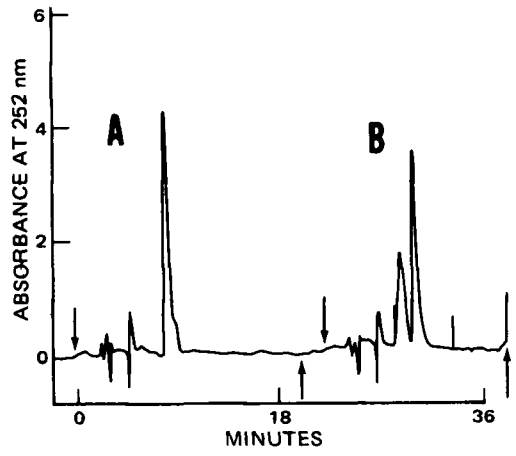

Figure 1—*Liquid chromatogram of I. Key: A, I injected directly; and B, I derived from spiked plasma. A down arrow (↓) indicates the start of integration, and an up arrow (↑) indicates the end of integration. The largest peak in each panel is I. The occasional shoulder on I is associated with impurities from dioxane.*

cm) and transferred to either 15-ml ground-glass conical centrifuge tubes or to all-glass evaporator cups[6]. A second and third extraction were performed similarly.

The pooled chloroform layers from each sample were dried under reduced pressure either in a water bath (45°) using a rotary evaporator or at 45° in a sample concentrator[6]. To the dried chloroform extracts was added 2 ml of chloroform, and the samples were redried. This step was repeated two times with 1 and 0.5 ml of chloroform, respectively. The final residues were transferred in 0.2 ml of dioxane to microsample vials, capped, and prepared for HPLC analysis.

Extraction Efficiency—Plasma from a local blood bank was used along with tritium-labeled and unlabeled I. The efficiency of extracting I from plasma was established using the following procedure. Known amounts of I and 0.01 μCi of radiolabeled I (14 μCi/μmole) were added to drug-free plasma (10 ml each) to achieve concentrations of I from 0.1 to 100 μg/ml. Aliquots (2.0 ml) of each plasma sample were transferred to thick-wall centrifuge tubes and stored frozen (−20°) until they were extracted. Samples ($n \geq 6$) at each concentration were analyzed in duplicate. Quantitation of I was accomplished by the relative response factors calculated from analysis of standards of I of known concentration. The linearity of the calibration curve for I was established over the concentration range of 0.1–100 μg/ml. Specificity for I was established using drug-free plasma.

Clinical Specimens—Written informed consent was obtained from two patients who participated in the institutionally approved I protocol. This protocol called for administration of I at 10 mg/m^2 by intravenous push followed immediately by a continuous infusion of I for 24 hr. Venous blood (5 ml) was collected in heparinized tubes at specified times and centrifuged for 2 min at 1000×*g*, and the plasma was removed and stored frozen until it was analyzed for I.

RESULTS AND DISCUSSION[7]

Figure 1A shows the elution profile of I (largest peak) directly injected into the high-pressure liquid chromatograph. The shoulder on the peak of I was observed with different batches of dioxane and was independent

[6] Brinkmann Instruments, Westbury, N.Y.

[7] The PROPHET computer system is sponsored by the Division of Research Resources of the National Institutes of Health (5).

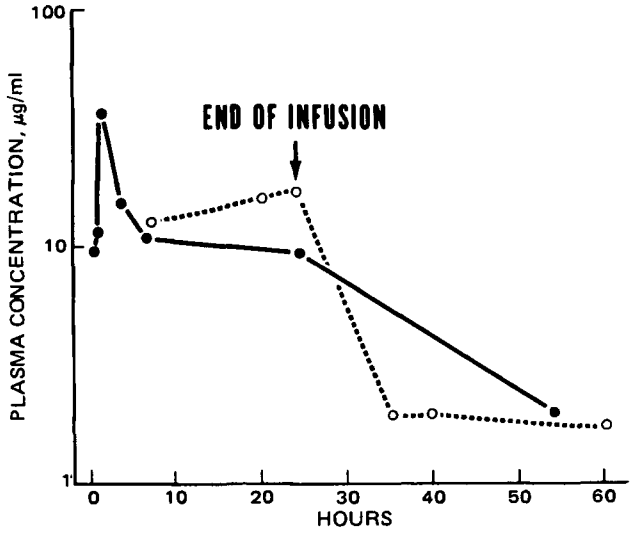

Figure 2—*Plasma levels of I. According to the protocol (written informed consent was obtained from the patients with an institutionally approved protocol), I was administered by a 24-hr continuous infusion at 180 (○) and 340 (●) mg/m^2 immediately after a 10-mg/m^2 short intravenous infusion (30 min).*

of the peak height of I. Figure 1B also shows a typical elution pattern of I (largest peak) derived from spiked plasma. To date, no interfering peaks from plasma have created a problem in the quantitation of I. The retention time of I in this system was 6.63 ± 0.04 min. A standard curve for I as a function of the peak area *versus* drug concentration (0.1–100 μg/ml) was linear with a correlation coefficient of >0.99 for six runs. The extraction efficiency for I over the concentration range of 0.5–90 μg/ml was 95.4 ± 3.8%, and this percentage was used in all recovery calculations. This range of drug concentrations is suitable for most clinical doses of the drug (>100 mg/m^2) and for measurements of I up to 48 hr or longer after drug administration.

Figure 2 shows two plasma curves for I as determined by this HPLC method. The more common wavelength detector (254 nm) can be used with somewhat reduced sensitivity for I (maximum sensitivity of 0.8 μg/ml). By increasing the proportion of methanol in the mobile phase to 75%, an analog of I, 4′-demethylepipodophyllotoxin-9-(4,6-*O*-thenylidene-β-D-glucopyranoside), can be quantitated in plasma by this method.

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