# Analysis of Procainamide Hydrochloride and Acecainide Hydrochloride in Rat Feed

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**Abstract** □ An extraction and GLC assay procedure was developed for quantitation of procainamide hydrochloride and acecainide hydrochloride in rat feed. 4-Amino-*N*-[2-(dipropylamino)ethyl]benzamide hydrochloride was synthesized and utilized as an internal standard. The assay has good precision and accuracy and was used to establish the stability of acecainide hydrochloride and procainamide hydrochloride in rat feed.

**Keyphrases** □ Procainamide hydrochloride—GLC analysis in rat feed □ Acecainide hydrochloride—GLC analysis in rat feed □ GLC—analysis, procainamide hydrochloride and acecainide hydrochloride in rat feed

The identification of acecainide (*N*-acetylprocainamide) as the major metabolite of procainamide in humans (1, 2) generated interest in acecainide as an antiarrhythmic agent and has required development of an assay for the simultaneous determination of these two drugs (3). Although sensitive spectrophotometric methods have been employed (4, 5), they do not exhibit the specificity of GLC (6, 7) and high-performance liquid chromatographic (8–11) assays reported for the analysis of these compounds in plasma and urine.

In accord with present regulatory trends, there also is a need to monitor accurately and ensure precise drug levels in chronic toxicity studies. Such studies are conducted at therapeutic and subtoxic drug concentrations and, thus, require at least two widely varying levels of drug determination.

The present study established a GLC procedure for accurately determining procainamide hydrochloride and acecainide hydrochloride concentrations in rat feed. A structural analog of procainamide, 4-amino-*N*-[2-(dipropylamino)ethyl]benzamide hydrochloride, is utilized as an internal standard to minimize extraction and assay variability.

## EXPERIMENTAL

**Materials**—Procainamide hydrochloride[1] and acecainide hydrochloride[2] were not <98% pure by nonaqueous titration. Elemental analysis and melting-point data also indicated the purity of the raw material. Diethyl ether was reagent grade. The rats were given a commercial rat food *ad libitum*[3].

The internal standard, 4-amino-*N*-[2-(di-*n*-propylamino)ethyl]-benzamide hydrochloride, was obtained through a synthetic scheme analogous to that employed for the synthesis of procainamide (12, 13). Recrystallization of the final product was effected from ethanol to provide white crystals in a 33% overall yield, mp 185–187°; chemical-ionization mass spectrum: M + 1, 264.

*Anal.*—Calc. for $C_{15}H_{26}ClN_3O$: C, 60.11; H, 8.68; N, 14.02. Found: C, 59.96; H, 8.74; N, 13.82.

**Instrumentation**—Assays were performed with a gas–liquid chromatograph[4] equipped with a flame-ionization detector. A 1.2-m × 2-mm i.d. glass column, packed with 3% OV-17 on 100–120-mesh Chromosorb W-HP, was maintained at 245 or 270° for procainamide or acecainide, respectively. The column inlet was maintained at 275°, while the detector temperature was 300°. The carrier gas was helium at a flow rate of 20 ml/min.

**Analysis of Rat Feed**—Standard solutions of procainamide hydrochloride and acecainide hydrochloride were prepared in water at 10.0 mg/ml. The internal standard solution was prepared in water at 4.0 mg/ml.

A 2.0-g homogeneous aliquot of rat feed containing drug was accurately weighed and added to 1.0 ml of the internal standard and 19 ml of water (pH 2) in a centrifuge tube. The contents were extracted and centrifuged. The water layer containing the drug and internal standard was transferred to a second tube. The solution was made basic (pH 12) with sodium hydroxide, sodium chloride (2 g) and ether (20 ml) were added, and the drugs were extracted into the ether layer. The contents were centrifuged, and the ether layer was drawn off for concentration and/or injection into the gas–liquid chromatograph (normally 2.0 μl).

Standard curves were constructed by spiking the drug-free rat feed with appropriate volumes of procainamide hydrochloride or acecainide hydrochloride standard solutions and following the procedure outlined. Peak area ratios of the drug to the internal standard were plotted against milligrams of drug per gram of feed.

## RESULTS AND DISCUSSION

In the analysis of acecainide and procainamide from rat feed, it was desirable to sample a representative quantity of drug in feed and to utilize an internal standard that would have physical properties similar to those of the test substances. Sampling of <1 g of feed could result in considerable assay variability caused by drug–feed mixing variability. Extraction of more than a few grams of feed requires large extraction vessels and considerable quantities of solvents and the internal standard. Extraction of samples and addition of the internal standard to an aliquot introduces assay variability caused by extraction differences.

The described procedure yielded small representative samples and permitted the use of a true internal standard. The use of the di-*n*-propyl derivative provided an internal standard with partitioning and chromatographic properties similar to those of acecainide and procainamide. The internal standard has a retention time between those of procainamide and acecainide and does not overlap with either test substance.

In the procainamide assay (column temperature 245°), procainamide has a retention time of ~2.8 min while the internal standard elutes at 4.0 min. With the acecainide analysis, the column temperature is 270°. Procainamide elutes with the solvent, the internal standard elutes at 1.8 min, and acecainide emerges at 3.5 min. This procedure is specific for acecainide and procainamide, with no interfering peaks present in the chromatogram.

The analysis of acecainide hydrochloride in rat feed was linear over a wide concentration range. Standards were determined in quadruplicate at 1.0, 2.0, 5.0, and 10.0 mg of drug/g of feed. The resulting plot had a correlation coefficient of 0.9990, an intercept of −0.004, and a slope of 0.115. The analytical range was extended by varying the concentration of the internal standard and the final volume of ether. Standards determined in duplicate at 10.0, 20.0, 30.0, and 40.0 mg of acecainide hydrochloride/g of feed gave a correlation coefficient of 1.000, an intercept of −0.142, and a slope of 0.145. In this example, 3.0 ml of the internal standard at 4.0 mg/ml was added to the feed, and the ether extract con-

[1] NAPP Chemical Co., Lodi, N.J.
[2] Ganes Chemical Co., New York, N.Y.
[3] Ralston-Purina, St. Louis, Mo.
[4] Model 5830, Hewlett-Packard, Avondale, Pa.

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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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Received December 14, 1979, from the *Departments of Oncology and Pharmacology R-71, School of Medicine, University of Miami, Miami, FL 33101.* Accepted for publication June 12, 1980.



**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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