

plastic surface, (b) followed by sorption of nitroglycerin to the plastic matrix by dissolution is in good agreement with the actual data. The model presented here should be useful for future investigations.

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High-Performance Liquid Chromatographic Determination of Verapamil in Plasma by Fluorescence Detection

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Abstract □ A rapid and highly sensitive method is reported for the quantitative determination of verapamil in plasma. Verapamil and its internal standard are extracted from alkalized plasma with heptane and then back-extracted into dilute sulfuric acid. An aliquot is injected directly into a high-performance liquid chromatograph, separated by reversed-phase chromatography, and quantified by a fluorescence detector. The procedure is suitable for the routine determination of verapamil in plasma in concentrations as low as 1 ng/ml.

Keyphrases □ Verapamil—high-performance liquid chromatography, fluorescence detection, canine plasma □ High-performance liquid chromatography—verapamil, canine plasma, fluorescence detection □ Antiarrhythmic agents—high-performance liquid chromatography, verapamil, canine plasma, fluorescence detection

Verapamil, 5-[(3,4-dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile (I), has been used for several years in Europe as an effective antiarrhythmic drug and is now undergoing clinical evaluation in the United States. Studies are underway in this laboratory to show the efficacy of verapamil in suppressing halothane-epinephrine arrhythmias (1).

Several analytical procedures have been reported for determining verapamil in plasma, including spectrophotometry (2), GLC with mass fragmentography (3), GLC (4), and high-performance liquid chromatography (HPLC) (5). None of these methods was considered completely satisfactory for reasons of simplicity, speed, and the ability to determine metabolites quantitatively. Therefore, an HPLC procedure was developed, and its application for the determination of verapamil in canine plasma following intravenous injection is reported here.

EXPERIMENTAL

Chemicals and Reagents—Verapamil¹ (I) and its internal standard, 4-[(3,4-dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylbutyronitrile² (II), were obtained as hydrochloride salts.

¹ Knoll Pharmaceuticals, Whippany, N.J.

² D517, Knoll AG, Ludwigshafen, West Germany.

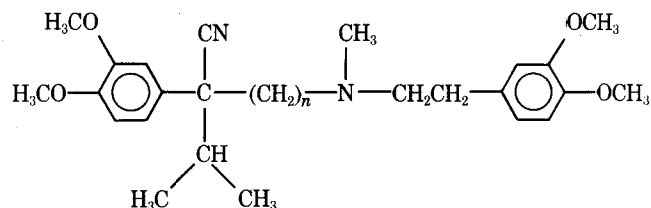
Glass-distilled heptane³ and HPLC grade acetonitrile⁴ were used. Sodium hydroxide (2 N), sulfuric acid (0.2 N), and pH 3.0 phosphate buffer (1 M) were prepared with deionized water.

HPLC—A liquid chromatograph⁵ was used with a fluorescence detector⁶. The column⁷ was C₁₈ reversed phase, and the eluting solvent was 45% acetonitrile–55% pH 3.0 potassium phosphate (0.1 M). The solvent was degassed prior to use by applying a vacuum. The flow rate was set at 1 ml/min. The detector settings were 203 nm for excitation with a 320-nm emission filter.

Procedure—To 0.5 ml of plasma were added 50 ng of II, 0.25 ml of 2 N NaOH, and 3 ml of heptane. The mixture was shaken mechanically for 15 min and then centrifuged. The organic layer was transferred to a 5-ml conical tube, and 50 μl of 0.2 N H₂SO₄ was added. The contents were mixed in a vortex mixer for 1 min and centrifuged, and a 10-μl sample was injected into the chromatograph.

RESULTS AND DISCUSSION

Standard curves were prepared routinely from spiked plasma samples containing 40, 100, and 250 ng of verapamil/ml. Peak height ratios were plotted as a function of the verapamil concentration added and were linear within this range. Intercepts usually passed through the origin. The lower limit of sensitivity of the assay was ~1 ng/ml, and this level was completely adequate for analyzing the samples in this study. Assay reproducibility and precision were evaluated by the repeated analyses of spiked plasma samples containing 100 ng of verapamil/ml. The coefficient of variation at this level was 4.6% (*n* = 12). The slopes of the standard curves from water or biological fluids were similar, indicating insignificant



I: *n* = 3

II: *n* = 2

³ Mallinckrodt Chemical Co., St. Louis, Mo.

⁴ MCB, Cincinnati, Ohio.

⁵ Model 330, Altex, Berkeley, Calif.

⁶ Model 970, Schoeffel Instruments, Westwood, N.J.

⁷ Ultrasphere ODS, Altex, Berkeley, Calif.

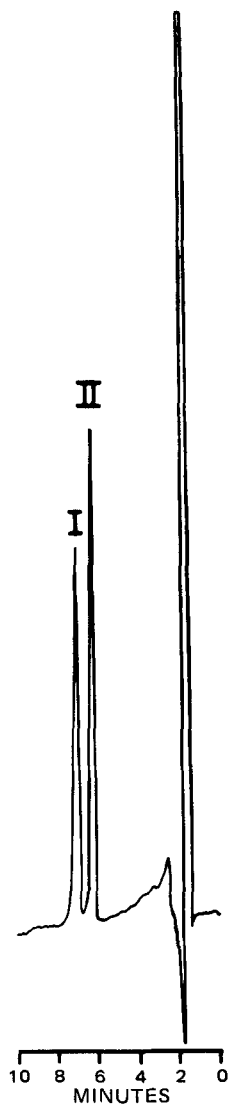


Figure 1—Chromatogram of the extract of a 0.5-ml plasma sample taken 8 min after the intravenous injection of 0.2 mg of verapamil/kg. Key: I, verapamil; and II, internal standard.

differences in the extractability of the compounds from aqueous solutions of different compositions.

A chromatogram of an extracted plasma sample from a dog that had received 0.2 mg of verapamil/kg iv 8 min previously is shown in Fig. 1. The verapamil concentration was 95 ng/ml.

Figure 2 shows a semilog plot of the concentration-time curve obtained after intravenous injection of 0.1 mg of verapamil/kg in a dog. The levels declined exponentially as fitted by the regression analysis program AUTOAN to a three-compartment open model according to:

$$C_x = Ae^{-\alpha t} + Be^{-\beta t} + Pe^{-\pi t} \quad (\text{Eq. 1})$$

where C_x is the plasma verapamil concentration; A , B , and P are the coefficients of the three compartments; and α , β , and π are the decay constants from these compartments. The terminal half-lives were 0.25, 6.5, and 129 hr, respectively. However, although the data from this experiment were compatible with a three-compartment model, it is not possible to state that three compartments exist for verapamil. Additional experiments of longer duration are required to reach statistically valid conclusions. The half-lives of the initial distribution phase ($t_{1/2\alpha}$, 0.25 hr) and for the apparent terminal phase ($t_{1/2\beta}$, 6.5 hr) were in agreement with previously reported values (6, 7).

The retention times of verapamil and II were 7 and 6 min, respectively. No endogenous compounds were present in plasma with the same elution times and fluorescence spectra.

A high first-pass effect was reported for patients receiving oral ver-

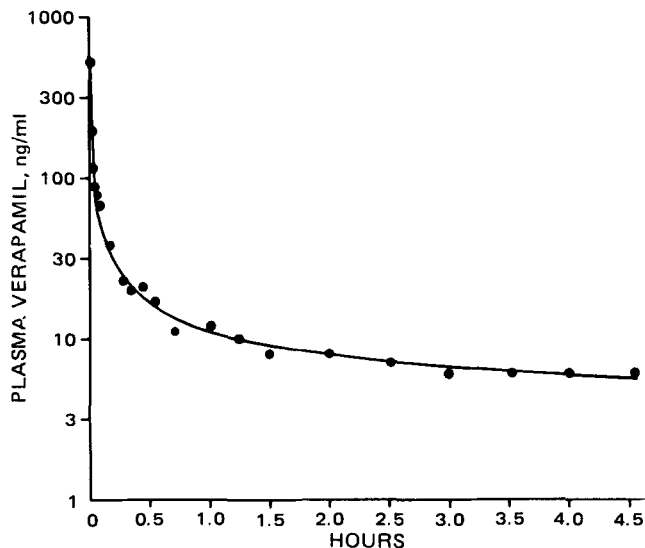


Figure 2—Change in plasma verapamil concentration with time after the intravenous injection of 0.1 mg/kg at time zero.

apamil (6). In the study reported here, verapamil was administered intravenously only; therefore, the presence of substantial amounts of metabolic products would not be expected. Throughout the study, no evidence of metabolic products was observed. Since these products would be more polar, their elution times would be expected to be less than the elution time of verapamil. Therefore, the use of heptane as the extracting solvent is extremely advantageous since there are no major peaks eluting prior to verapamil that may possibly obscure the presence of metabolic products. While authentic metabolites have not been tested in this chromatographic system, it is reasonable to expect that they would elute ahead of verapamil.

Other studies are being planned to evaluate the pharmacokinetics of verapamil and the individual metabolic products following oral administration. A discrepancy exists between the duration of the electrophysiological and hemodynamic effects of verapamil and its concentration in plasma. The possibility that this discrepancy may be related to the concentration of metabolites has been proposed (8), and the method described here may be of value in resolving this problem.

The ultimate objectives in undertaking pharmacokinetic metabolic and disposition studies are to predict disposition in individual patients and to optimize doses and schedules rapidly. The development of a highly sensitive and specific analytical procedure for measuring verapamil in plasma should enable more detailed and longer term clinical disposition studies than have been reported previously.

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