Journal of Chromatography, 170 (1979) 385–390
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 11,516

RAPID HIGH-PRESSURE LIQUID CHROMATOGRAPHIC ANALYSIS OF VERAPAMIL IN BLOOD AND PLASMA

SANDRA R. HARAPAT and ROBERT E. KATES*

Pharmacokinetics Laboratory, Cardiology Division, Stanford University School of Medicine, Stanford, Calif. 94305 (U.S.A.)

(Received October 3rd, 1978)

SUMMARY

A high-pressure liquid chromatographic assay procedure has been developed for verapamil in blood or plasma. A paired-ion solvent system with a reversed-phase column is employed. The procedure is specific for verapamil and the retention times of the major metabolites are identified. This procedure is sensitive to a lower blood concentration of 1 ng/ml and standard curves were found to be linear up to the highest concentration tested, 500 ng/ml. Several drugs were tested for interference with the assay, but none were found to cause any problems. The procedure is simple, rapid and permits the analysis of up to 25 samples per day.

INTRODUCTION

Verapamil is an effective antiarrhythmic agent which exerts its effect through inhibition of slow inward calcium currents[1,2]. Clinically, it is most useful in the control of supraventricular tachyarrhythmias[3,4]. As with all clinically useful antiarrhythmic drugs, the pharmacological effects of verapamil appear to be related to its concentration in the plasma. In order to develop a scientific basis for the design of rational dosage regimens, it is necessary to understand the pharmacokinetics and the plasma level–effect relationship for this drug. To facilitate such studies, one must have a reliable routine analytical procedure.

Current methods for analyzing verapamil in biological fluids include a spectrofluorometric procedure[5] and a mass fragmentographic procedure[6] which utilizes stable isotope labelled drug as internal standard. The spectrofluorometric method has a lower limit of sensitivity of only 1 μg/ml and is not adequately specific. The mass spectrometric procedure, while adequately sensitive and specific, is not applicable for routine monitoring of large numbers of samples due to its complexity and the need for highly sophisticated equipment. In order to facilitate routine monitoring of verapamil concentrations in human blood samples, a simple rapid high-pressure

* To whom correspondence should be addressed.

liquid chromatographic procedure was developed. The method described here, which employs a paired-ion reversed-phase system, is sensitive to a concentration of 1 ng/ml of verapamil per sample and provides a good separation of verapamil from its two major metabolic products (see Fig. 1).

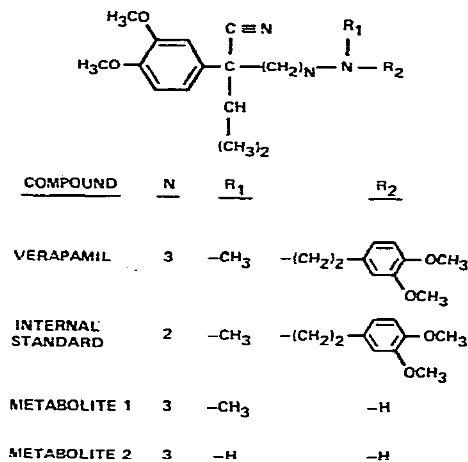


Fig. 1. The chemical structures of verapamil, the internal standard used in the analysis, and the two major metabolites of verapamil.

EXPERIMENTAL

Chemical and reagents

Verapamil (α-isopropyl-α-[(N-methyl-N-homoveratryl)-γ-aminopropyl)]-3,4-dimethoxy-phenylacetronitrile hydrochloride), the two N-dealkylated metabolites, and the internal standard (see Fig. 1) were generously provided by Knoll Pharmaceutical Co. (Whippany, N.J., U.S.A.). The paired-ion reagent, 2-propanesulfonic acid sodium salt was obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.). Glass-distilled methanol was obtained from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.). All other solvents were of reagent grade.

Instrumentation

A Varian Model 8500 high-pressure liquid chromatographic system using a Varian stop-flow injector port was employed. The column used was a Varian Micro-Pack MCH-10 reversed-phase column (25 cm × 2 mm I.D.). The flow-rate was adjusted to 60 ml/h which developed a pre-column pressure of about 190 atm (2800 p.s.i.). The detector was a Schoeffel 970 fluorometer which was set for excitation at 203 nm with a 320 nm emission cutoff filter. A detector range setting of 0.1 μA was used together with a Varian Model 9176 dual-pen recorder set at 5 and 10 mV outputs.

Mobile phase preparation

The mobile phase was prepared by mixing aqueous and methanolic solutions of the paired-ion reagent. The aqueous solution was prepared by dissolving 1.3 g of

2-propanesulfonic acid sodium salt (0.01 mole) in 1 l of water and adjusting the pH to 3.9 with reagent-grade glacial acetic acid (approximately 1.5 ml). This solution was then filtered through a Whatman No. 2 filter paper using vacuum. The methanolic solution was prepared by adding the same quantity of paired-ion reagent and acetic acid to 1 l of methanol. The mobile phase was then prepared by mixing the two solutions in a ratio of 1:1.56 (methanol–water).

Extraction procedure

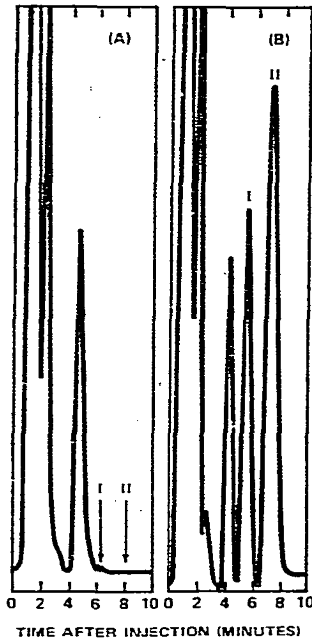
Whole blood or plasma samples (0.1–1.0 ml) were pipetted into PTFE-lined screw-capped tubes and 100 μl (16 ng) of an aqueous solution of the internal standard, 20 μl of 2 N NaOH and 5 ml of diethyl ether were added. For samples containing more than 100 ng/ml verapamil, larger amounts of internal standard were employed. The samples were extracted by gently tilting for 10 min and centrifuged. The aqueous layer was frozen by placing the tube in a dry ice–acetone bath and the ether was decanted into a clean tube with an elongated cone at its base of approximately 50 ml capacity. A 20-μl aliquot of 0.25 N H_2SO_4 was then added to the ether. The tube was capped, extracted on a Vortex mixer for 1 min and centrifuged. The acidic aqueous phase was injected into the chromatograph using a 50-μl syringe.

RESULTS AND DISCUSSION

The retention times for verapamil, the primary and secondary amine metabolites, and the internal standard were 7.8, 3.2, 2.7 and 6.2 min, rexpectively. The column temperature was not controlled, but generally stayed around 30°. While changes in temperature did affect the retention times, a 3° fluctuation was not found to cause a significant problem.

A chromatogram of an extracted blank plasma sample is shown in Fig. 2A. A peak was observed with a retention time of 4.5 min, but this did not interfere with the verapamil or internal standard peaks. The rapidly eluting plasma constituents which came off just after the solvent front, made it difficult to quantitate the two major verapamil metabolites. Fig. 2B is a chromatogram of an extracted plasma sample to which had been added 25 ng of verapamil and 16 ng of the internal standard. While the major metabolites cannot be quantitated using the conditions described, they are sufficiently separated from both verapamil and the internal standard that they do not interfere with the quantitation of the unchanged drug. Since patients receiving antiarrhythmic drug therapy often are given other medications concurrently, it is necessary to evaluate the possibility of assay interference from other compounds. Several drugs were added to plasma samples in quantities representative of therapeutic or higher concentrations. These were then injected into the chromatograph. The following drugs were evaluated: propranolol, quinidine, procainamide, chlorthiazide, prazosin, furosemide, and hydralazene. None were found to cause interference with the analysis of verapamil.

Standard curves were prepared by adding known amounts of drug to blank plasma or blood samples and determining the peak height ratios (verapamil/internal standard). The peak height ratios were then plotted as a function of the concentration of drug added. The verapamil standard curves were linear over a range of from 1 to 500 ng/ml added, and extrapolated through the origin. A typical curve, from 5



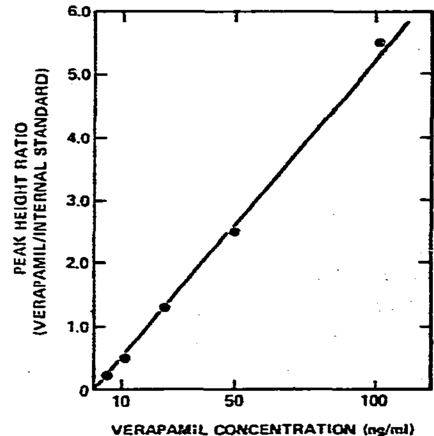


Fig. 2. Chromatograms of extracts of a 1-ml blank plasma sample (A) and a 1-ml plasma sample containing 16 ng of internal standard (I) and 25 ng of varapamil (II). The peak eluting at 4.5 min was an unidentified plasma constituent. The detector range for both chromatograms was 0.1 μA.

Fig. 3. A typical calibration curve for verapamil extracted from 1 ml plasma. The coefficient of variation for the normalized peak height ratio was 5.2%.

to 100 ng/ml, is shown in Fig. 3. The peak height ratios do not differ between extractions from whole blood or plasma. The average coefficient of variation from four standard curves was 5.2% (see Table I). The daily variation in the slope of the regression line, however, had a coefficient of variation of 3.8%.

The reproducibility of the procedure was evaluated by analyzing replicate plasma samples to which known concentrations of verapamil had been added. The results of this evaluation are shown in Table II. Good reproducibility was found and the average coefficient of variation was 3.7%. To facilitate analysis of samples of high concentration without preparing a high range standard curve, the influence

TABLE I

CALIBRATION CURVE DATA

C.V. = coefficient of variation.

Sample	*Range (ng/ml)*	*N*	*% C.V. in normalized peak height ratio*
Blood	5–100	5	5.9
Plasma	1–50	6	6.6
Plasma	5–100	5	6.4
Plasma	5–100	5	5.2
Plasma	50–500	4	3.5
		Average	5.5

TABLE II

REPRODUCIBILITY AT A GIVEN PLASMA CONCENTRATION

C.V. = coefficient of variation.

Concentration (ng/ml)	*N*	*% C.V.*
5	7	3.5
25	7	3.5
100	7	4.2
	Average	3.7

of varied sample size was evaluated. Spiked plasma samples from 0.1 to 1 ml were analyzed. It was found that sample size did not affect the relative extraction efficiencies of verapamil or the internal standard.

While similar results are obtained if one adds known concentrations of drug to a plasma or whole blood sample, it cannot be inferred that the partitioning of verapamil between plasma water and red blood cells is uniform. It has been reported that verapamil in the plasma is about 90% bound to albumin[7]. One would therefore expect to find a higher concentration of verapamil in a plasma sample from a patient, than in whole blood. To evaluate the distribution of verapamil in whole blood, known concentrations of verapamil were added to blood samples from five different patients. Aliquots of blood were analyzed and the remainder was centrifuged and the plasma analyzed. The results are shown in Table III. The average ± standard deviation (S.D.) blood–plasma partition ratio was 0.63 ± 0.05. While the plasma concentration is much greater than the whole blood at the concentration evaluated, it has not been determined whether this is concentration dependent.

The procedure reported here has sufficient sensitivity for both therapeutic monitoring and pharmacokinetic studies. It is specific for verapamil and could be modified to permit quantitation of the two major metabolites of verapamil. The method is relatively simple and rapid allowing the analysis of 25 samples in a day.

TABLE III

BLOOD/PLASMA PARTITION RATIO

Sample No.	*Blood concentration (ng/ml)*	*Blood/plasma*
1	53	0.58
2	65	0.56
3	64	0.68
4	54	0.65
5	58	0.67
	Average	0.63

ACKNOWLEDGEMENT

This work was supported in part by NIH Grant No. HL-21221.

REFERENCES

1 M. R. Rosen, J. P. Ilvento and C. Merker, *Amer. J. Cardiol.*, 33 (1974) 166.
2 M. Kohlhardt, B. Bauer, H. Drause and Al. Fleckenstein, *Experientia*, 28 (1972) 288.
3 D. Krikler and R. Spurrell, *Postgrad. Med. J.*, 50 (1974) 447.
4 J. Vohra, D. Hunt, J. Stuckey and G. Sloman, *Brit. Heart J.*, 36 (1974) 570.
5 R. G. McAllister and S. M. Howell, *J. Pharm. Sci.*, 65 (1975) 431.
6 B. Spiegelhalder and M. Eichelbaum, *Drug Res.*, 27 (1977) 94.
7 M. Schomerus, B. Spiegelholder, B. Dtieren and M. Eichelbaum, *Cardiovasc. Res.*, 10 (1976) 605.