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Note

High-performance liquid chromatographic analysis of verapamil

II. Simultaneous quantitation of verapamil and its active metabolite, norverapamil

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Several analytical procedures have been published recently for measuring the concentration of verapamil in plasma and urine [1—3]. These reports discuss the separation of verapamil from its N-dealkylated metabolites (Fig. 1). However, no procedure has been reported for measuring the N-demethylated metabolite, norverapamil which has been reported to accumulate in the plasma of patients after oral administration [4]. This metabolite apparently does not accumulate significantly in the plasma of patients receiving single intravenous doses of verapamil, however it does accumulate to concentrations equal to or greater than those of verapamil during oral administration. Norverapamil has been reported to be about 20% as potent a vasodilator as verapamil when administered intra-arterially to dogs, but appears to have no significant effect on AV nodal conduction [5].

While previously developed assay procedures for verapamil are very useful for measuring concentrations of the drug in biological fluids following short-term or acute administration, it remains to be seen if these procedures provide adequate separation of verapamil from norverapamil. Due to the close structural similarity between verapamil and norverapamil and the high concentrations of the metabolite observed in plasma of patients on chronic oral therapy, it is difficult to adequately separate these two compounds by our previously reported high-performance liquid chromatographic (HPLC) procedure [3], without substantially slowing down the chromatography. This modification would greatly restrict the number of samples which could be analyzed daily and a loss of sensitivity would also occur. Therefore an alternative chromatographic procedure was developed to facilitate separation and simultaneous quantitation of

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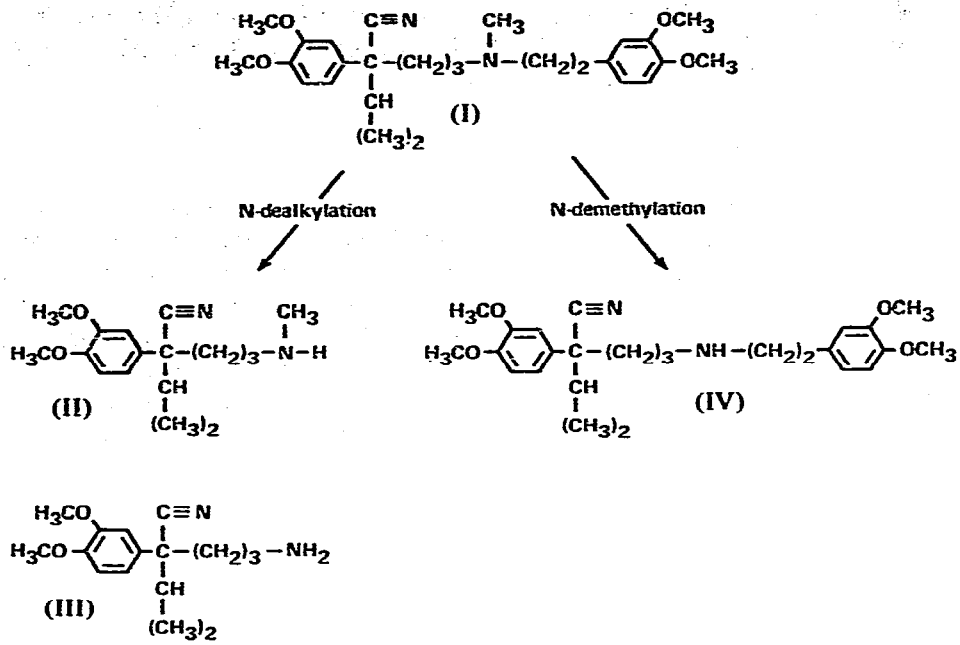

Fig. 1. Metabolic scheme for verapamil (I). The N-demethylated compound (IV) is the major active metabolite, norverapamil.

verapamil and norverapamil in human plasma. The method described here is a reversed-phase HPLC procedure which has a lower limit of sensitivity of 3 ng/ml for both verapamil and norverapamil when analyzed simultaneously.

EXPERIMENTAL

Verapamil, the two N-dealkylated metabolites and the internal standard (α-isopropyl-α-[(N-methyl-N-homoveratryl)-β-aminoethyl]-3,4-dimethoxyphenylacetonitrile hydrochloride) were obtained as HCl salts from Knoll Pharmaceutical Company (Whippany, N.J., U.S.A.) and norverapamil HCl was supplied by Knoll AG (Ludwigshafen, G.F.R.). Glass distilled acetonitrile was purchased from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.). All other chemicals and solvents were of reagent grade.

The extraction procedure and instrumentation as well as internal standard remain the same as previously reported [3]. Changes have been made in the chromatography to facilitate the separation of verapamil and norverapamil within reasonable time limits. The column used was a 10-μm particle size μBondapak C_{18} (30 cm × 3.9 mm I.D.) (Waters Assoc., Milford, Mass., U.S.A.). The mobile phase consisted of acetonitrile—dilute H_2SO_4 (29:71). The dilute acid (ca. 0.004 *N*) was prepared by adjusting the pH of distilled water to 2.4 with dropwise addition of concentrated H_2SO_4. The flow-rate of mobile solvent was 150 ml/h which developed a precolumn pressure of 108 atm (1500 p.s.i.).

A blinded study was carried out with plasma samples to which were added known concentrations of verapamil, norverapamil, and the two N-dealkylated metabolites. They were analyzed for verapamil and norverapamil concentra-

tions by the method reported here without knowledge of the actual concentrations. The samples were initally spiked in the laboratories of Knoll AG. The results were then compared with the actual values.

RESULTS AND DISCUSSION

The retention times for verapamil, the internal standard, norverapamil and the two N-dealkylated metabolites are listed in Table I. With the conditions used, the two N-dealkylated metabolites were not completely separated from one another. These could be resolved if one either decreased the percentage of the acetonitrile in the eluting solvent or slowed the flow-rate considerably. These changes would result in much longer retention times with subsequent loss of sensitivity for verapamil. Since these two N-dealkylated metabolites are of very low pharmacological activity, compared to verapamil, their quantitation was not considered important.

TABLE I

RETENTION TIMES OF VERAPAMIL AND METABOLITES

Compound	Retention time (min)
Metabolite III	3.2
Metabolite II	3.5
Internal standard	10.5
Norverapamil	11.7
Verapamil	13.0

Chromatograms of an extracted blank plasma sample (A), injected standard solutions (B), and an extracted plasma sample from a patient on long-term oral verapamil therapy (C) are shown in Fig. 2. The peak in the patient sample (C) which eluted just prior to the internal standard did not correspond to any of the three metabolites being measured or the other medications which the patient was concurrently receiving and was not identified. This peak has not been observed in plasma samples from other patients. The concentrations of verapamil and norverapamil in the patient sample were 350 ng/ml and 245 ng/ml, respectively. This patient was taking 80 mg of verapamil HCl four times daily and the sample was drawn just after administration of a morning dose. Other patient samples which have been analyzed have had concentrations of norverapamil which were in some cases greater than those of verapamil.

Standard curves were prepared by adding known amounts of verapamil, norverapamil and 25 ng of internal standard to blank plasma, analyzing the samples, and determining the verapamil and norverapamil to internal standard peak height ratios. The curves were linear from 2 to 500 ng/ml for both verapamil and metabolite. A standard curve for both compounds over a range of 0 to 115 ng/ml is shown in Fig. 3. At a given concentration, the peak height of verapamil was greater than norverapamil, despite the fact that the latter eluted from the column first. This suggests that verapamil is more fluorescent than the metabolite at the wavelengths used. The slopes of the standard curves are 0.356 and 0.296 for verapamil and norverapamil, respectively.

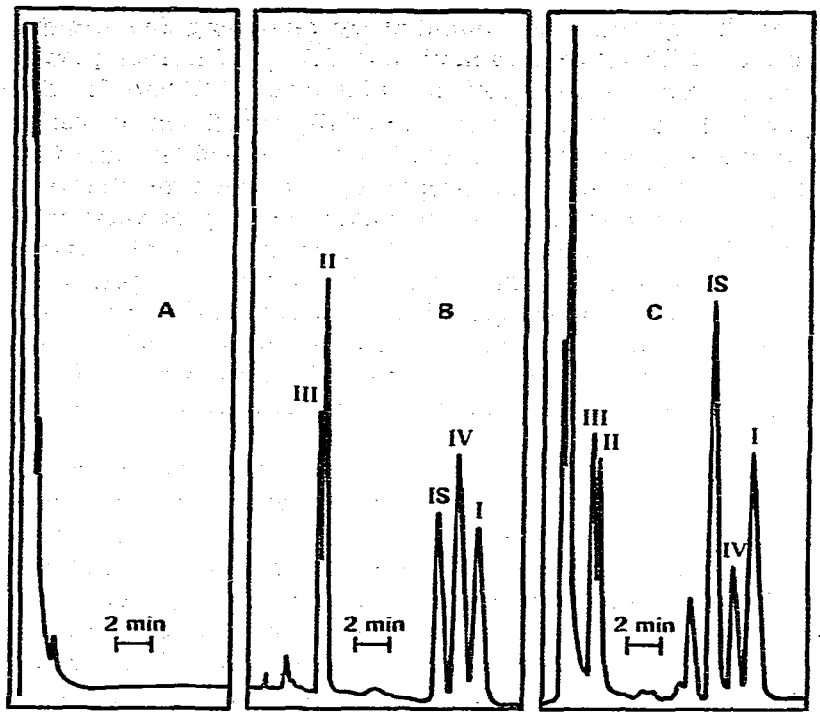

Fig. 2. Chromatograms of an extracted blank plasma sample (A), a mixture of verapamil (I), norverapamil (IV), internal standard (IS), and the two N-dealkylated metabolites (II, III) (B), and an extracted plasma sample from a patient receiving chronic oral verapamil (C).

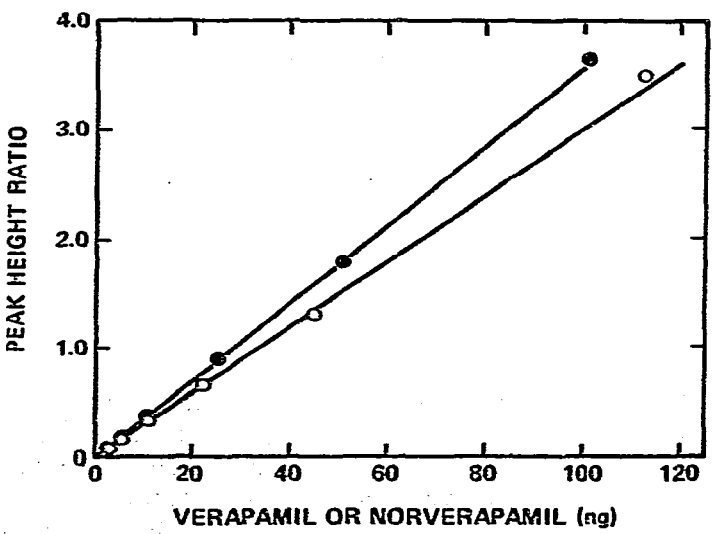

Fig. 3. Calibration curves for verapamil (•) and norverapamil (○). The coefficients of variation for the normalized peak height ratios were 3.0% and 3.7% for verapamil and norverapamil, reespectively.

The reproducibility of the system was evaluated by analyzing five plasma samples to which were added 25 ng of verapamil and 22 ng of norverapamil. The coefficient of variation for the verapamil samples was 5.1% and for the norverapamil samples 3.5%. These values are similar to the coefficient of variation observed with the previously reported procedure [3]. To facilitate analysis of samples of high concentration without preparing a high-range standard curve, the influence of varied volume size was evaluated. Ten ng of verapamil and 11 ng of norverapamil were added to varied volumes of blank plasma ranging from 100 μl to 1 ml. Internal standard was added and the samples analyzed. The coefficients of variation for verapamil and norverapamil were 3.1% and 5.1%, respectively. The extraction efficiency was determined for norverapamil by comparing the peak heights of chromatograms from extracted and directly injected samples of the metabolite. The extraction efficiency was greater than 60%.

The results of the comparison samples which were analyzed by the HPLC procedure reported here are summarized in Table II. Verapamil and norverapamil were analyzed simultaneously by the HPLC procedure. The samples containing the low verapamil concentrations contained the lower norverapamil concentrations and in similar manner the medium- and high-range verapamil samples contained the medium- and high-range norverapamil concentrations, respectively. The deviations of the results are expressed as percent of the spiked value. The overall percent deviation for the 17 verapamil and norverapamil analysis were 4.6 and 9.8, respectively. Correlations for the two methods were good with $r = 0.99$ for both compounds.

TABLE II

RESULTS OF BLINDED ANALYSIS STUDY

Range (ng/ml)	*N*	Average percent deviation
Verapamil		
1—10	6	4.7
10—100	5	6.1
100—500	6	3.2
Norverapamil		
1—20	6	22.1
50—200	5	6.6
200—1000	6	0.1

Interference from other drugs was evaluated by adding various drugs to plasma in quantities representative of therapeutic or higher concentrations. These were then extracted and injected into the chromatograph. The following drugs were evaluated: digoxin, theophylline, disopyramide, propranolol, quinidine, procainamide, chlorthiazide, prazosin, furosemide, and hydralazine. None of these compounds interfered with the analysis of verapamil or norverapamil.

The method reported here differs from our previously reported procedure in that this method allows the simultaneous quantitation of verapamil and its major active metabolite, norverapamil. The previous method is sufficient for analysis of samples from patients receiving single intravenous bolus doses where

the metabolite does not accumulate to sufficient concentrations to interfere with the assay. These samples could also be analyzed at a more rapid rate with the present method by increasing the percent of acetonitrile in the mobile phase. The method reported here, however, provides a better separation of verapamil from norverapamil which is sufficient to allow quantitation of both species.

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