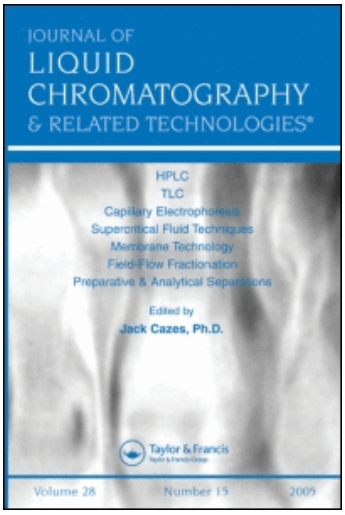

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Determination of Verapamil and Norverapamil in Serum by High-Performance Liquid Chromatography

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DETERMINATION OF VERAPAMIL AND NORVERAPAMIL IN SERUM BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple, rapid, sensitive and specific high-performance liquid chromatographic method is developed for the determination of verapamil and its active metabolite norverapamil in human serum. The sample (100 µl) is vortex-mixed with methanol (200 µl) and after centrifugation 100 µl of the supernatent is used for the analysis of a C_8 reverse-phase column (10 cm x 5 mm) with methanol - 0.1 M ammonium acetate (55:45 v/v) as the mobile phase. The compounds are detected fluorimetrically with a detection limit of 5 µg/l using an excitation wavelength of 278 nm and an emission wavelength of 320 nm. The method is applicable to pharmacokinetic studies and to therapeutic monitoring of the drug and its active metabolite.

INTRODUCTION

Verapamil (Figure 1) is an anti-anginal, anti-hypertensive and anti-arrhythmic agent which is at present undergoing clinical evaluation in the United States although it has been used for several years in Europe. The compound is extensively

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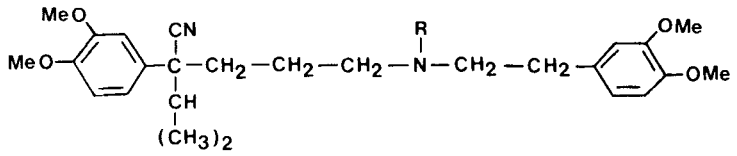

Figure 1. Structural formulae of verapamil (R = CH_3) and norverapamil (R = H).

transformed into the N-dealkylated, N-demethylated and O-demethylated metabolites. Norverapamil (Figure 1), the N-demethylated metabolite is pharmacoligically active and may accumulate to serum level greater than its parent compound.

Several methods have been described for the determination of verapamil and norverapamil. The spectrofluorimetric method (1), unable to separate verapamil from its metabolites, is of limited use. Gas liquid chromatographic procedures (2,3) required large sample volumes and tedious sample preparation steps prior to analysis. Recently high performance liquid chromatography (HPLC) have been used (4,5,6,7) for the determination of verapamil and norverapamil in plasma. However, except for the method of Cole et al. (7), complicated extraction procedures are still being used.

We are interested in developing a sensitive and specific assay for verapamil and its metabolites for use in therapeutic monitoring and for pharmacokinetic and disposition studies.

This paper describes an improved HPLC method for the determination of verapamil and norverapamil in human serum. A 10 cm x 5 mm MOS-Hypersil column (5 µm spherical silica chemically bonded with dimethyl octyl groups) eluted with methanol - 0.1 M ammonium acetate (55:45 v/v) effectively resolved verapamil and norverapamil from the other metabolites including the dealkylated metabolites D617 and D620. A fluorescence detector provides the necessary sensitivity and specificity.

EXPERIMENTAL

Materials and Reagents

Verapamil, norverapamil and the N-dealkylated metabolites D617 and D620 were from Abbott Labs (Queenborough, U.K.). Ammonium acetate was AnalaR grade from BDH (Poole, U.K.). HPLC grade methanol was from Rathburn Chem. Ltd. (Walkerburn, U.K.).

Sample Preparation

Serum (100 µl) was vortex-mixed with ice-cold methanol (200 µl) for 30 sec. in a small glass vial and then centrifuged for 3 min. at 1000 g to remove the precipitated serum proteins. Two 100 µl portions of the supernatant were used for duplicated analysis. To avoid contaminations from plasticisers only glass vials should be used.

High Performance Liquid Chromatography

A Pye-Unicam (Cambridge, U.K.) LC3-XP solvent delivery system coupled to a Perkin-Elmer (Beaconsfield, U.K.) LS-3 fluorescence spectrometer was used. Sample injection was via a Rheodyne 7125 injector fitted with a 100 µl sample loop.

A 10 cm x 5 mm MOS-Hypersil column (5 µm spherical silica chemically bonded with dimethyl octyl groups) was used for the analysis. The mobile phase was 55% methanol in 0.1 M ammonium acetate and was thoroughly degassed before use.

The flow rate was 1 ml/min. The detector was set at an excitation wavelength of 278 nm and an emission wavelength of 320 nm.

Calibration Curve

This was constructed from human serum samples spiked with verapamil and norverapamil or from aqueous solutions containing

the drugs in the range 10 - 100 µg/l and was linear within this range. There was no significant difference in the curves plotted from serum and aqueous standards.

RESULTS AND DISCUSSION

The separation of verapamil, norverapamil, D617 and D620 is shown in Figure 2. The choice of a C_8 reversed-phase column (MOS-Hypersil) with methanol - 0.1 M ammonium acetate (55:45 v/v) as the mobile phase effectively separated the compounds in about 15 min. Other reversed-phase packings (C_1, C_2, C_{18}) are also suitable with the appropriate adjustment of the eluent. The presence of ammonium acetate is essential for complete separation of the compounds, and replacing it with water or acetate buffers led to a loss of resolution. The concentration of the ammonium

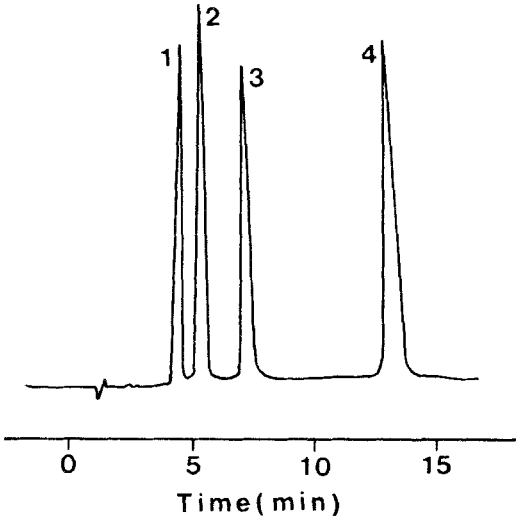

Figure 2. Separation of D620 (1), D617 (2), norverapamil (3) and verapamil (4). Column, MOS-Hypersil (10 cm x 5 mm); mobile phase, 55% methanol in 0.1 M ammonium acetate; flow rate, 1 ml/min.

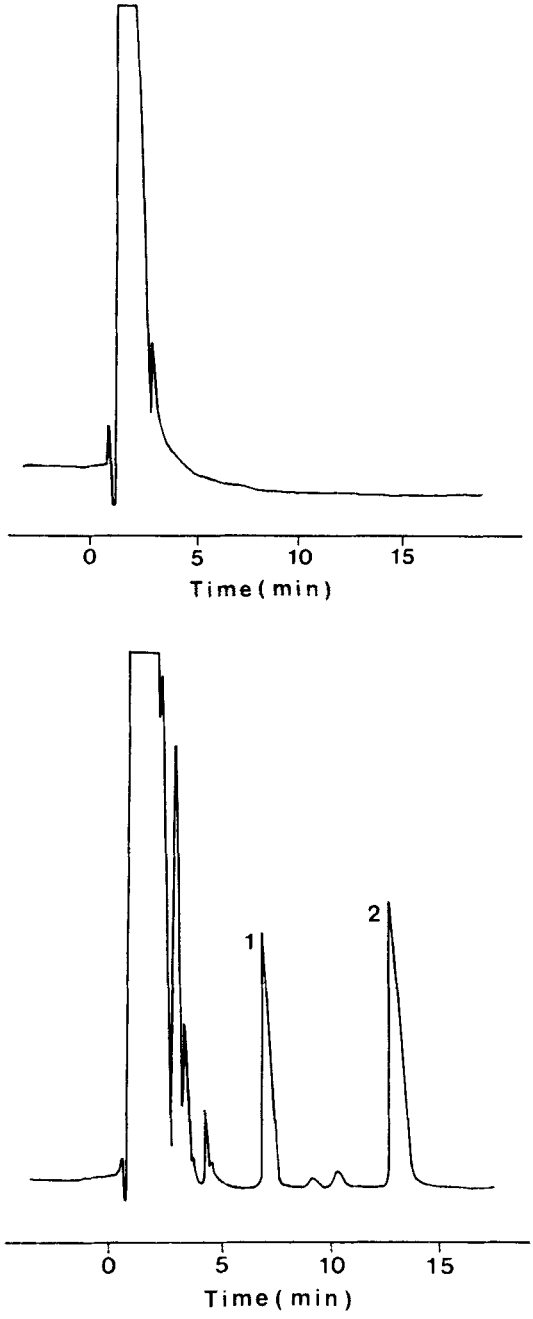

Figure 3. Separation of norverapamil (1) and verapamil (2) in serum. (a) Blank; (b) of a patient treated with verapamil. HPLC conditions as in Figure 2.

acetate solution is not critical provided it is above 0.05 M. It is not necessary to use columns longer than 10 cm unless the separation of minor metabolites is also required.

The use of large sample volume and complicated extraction procedures (4,5,6) is also unnecessary. The method described here needs only 100 µl of serum for a duplicated analysis. The drug and its metabolites can be simply and effectively extracted into methanol which also precipitated the serum proteins. The supernatant can then be injected after a quick centrifugation. The co-precipitation of verapamil and norverapamil with the proteins was not a problem. This is demonstrated by their complete recovery in sera spiked with the drugs.

Figure 3 shows the chromatograms of a drug free serum sample (a) and a sample from a patient treated with verapamil (b). No interference from endogenous serum constituents was observed.

The reproducibility and precision of the method were evaluated by repeated analysis of a serum sample containing 100 µg/l of verapamil and norverapamil. The coefficient of variation was 3.5% (n = 15) and 3.2% (n = 15) respectively.

The use of fluorescent detection makes the method both specific and sensitive. A detection limit of 5 µg/l can be achieved. This is sufficient for pharmacokinetic and disposition studies and for the therapeutic monitoring of the drug and its active metabolites.

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