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Note

Determination of mepamil in human plasma by reversed-phase high-performance liquid chromatography with fluorescence detection

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Mepamil (MEP), 2-(2-methylphenyl)-2-isopropyl-5-[N-2-(3,4-dimethoxyphenyl)ethyl-N-methylamino]valeronitrile, is an original Czechoslovak compound that appears to have an antianginal and antiarrhythmic effect. As MEP is an original compound, we based our analysis strategy on the previously described determinations of verapamil (VEP) [1–12]. In the literature there are also accounts of gas chromatographic methods [13–16] for the determination of VEP. The high-performance liquid chromatographic (HPLC) methods are distinguished by their simultaneous determination of different metabolites. The limit of detection is satisfactory, because the plasma levels of VEP after the administration of therapeutic doses are in the range 10–500 ng/ml of plasma [3]. On the other hand, the plasma levels of MEP after a single oral administration of an 80-mg dose (the first clinical phase) are in the range 1–50 ng/ml of plasma.

This paper describes a reversed-phase HPLC method with fluorescence detection for the quantitative determination of plasma levels of MEP. The method also enables the determination of VEP after replacement of an examined compound and internal standard.

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EXPERIMENTAL

Instrumentation

Chromatography was performed on a component system consisting of a Model SP 8770 pump (Spectra Physics, San Jose, CA, U.S.A.), a Model FS 970 fluorescence detector (Kratos, Ramsey, NJ, U.S.A.), a Model SP 4100 integrator (Spectra Physics) and a Model 7125 injector (Rheodyne, Cotati, CA, U.S.A.). Chromatographic separation was carried out on a 250 mm×4.6 mm I.D. Supelcosil LC-18 column (5 μm particle size) with a guard column (50 mm×4.6 mm I.D. LC-18, 40 μm particle size). The column and guard column were supplied from Supelco (Bellefonte, PA, U.S.A.).

Materials

MEP and VEP were supplied from the Research Institute for Pharmacy and Biochemistry (Prague, Czechoslovakia). Hexane for spectroscopy was purchased from Merck (Darmstadt, F.R.G.). Triethylamine was obtained from Fluka (Buchs, Switzerland). Phosphoric acid, sodium dihydrogenphosphate, borax and sodium hydroxide were supplied from Lachema (Brno, Czechoslovakia) in quality p.a. Methanol was rectified. Water was redistilled.

Extraction

A 50-μl volume of the internal standard solution (0.8 μg VEP per ml 50% methanol) was placed in a 10-ml silanized glass centrifuge tube (silanization was performed according to Sioufi and Pommier [17]) to which 2 ml of plasma, 1 ml of borate buffer (0.5 *M*, pH 10) and 4.5 ml of hexane were added. The tube was shaken mechanically for 15 min and centrifuged at 2500 *g* for 5 min. Then 4 ml of the organic phase were transferred to a conical silanized tube and evaporated to dryness. The residue was reconstituted with 100 μl of mobile phase, and 20 μl were injected into the chromatograph.

Calibration curves

Calibration samples were prepared by introducing 100 μl of the solution of MEP in 50% methanol at concentrations of 0, 1, 5, 10, 25 and 50 ng/ml of plasma. The mixtures were processed as described above.

Chromatographic conditions

The mobile phase was methanol–triethylamine–5 m*M* sodium dihydrogenphosphate (65:1:34, v/v), and the pH was adjusted to 3 with phosphoric acid. The flow-rate was 1 ml/min and the column temperature was 40°C. The excitation wavelength of the fluorescence detector was fixed at 195 nm and analysis was performed with no filters. The detector range was set at 0.05–0.1 μA and the time constant was maintained at 4 s.

RESULTS AND DISCUSSION

The retention times of VEP and MEP were 4.8 and 6.5 min, respectively, and the capacity factors were 0.78 and 1.41, respectively. The analysis time was 10 min. Better accuracy was obtained by using peak heights for analysis. The intra-day reproducibility of the calibration curves was determined with five different plasma samples (Table I). It corresponds to the equation C (ng/ml of plasma) $= 0.17 + 26.88(H/H_{IS})$ with a correlation coefficient $r = 0.9979$; the curve was linear over the above concentration range. The plasma amount was reduced if the concentration of MEP was above 50 ng/ml of plasma. It is possible to use one-point calibration in this concentration range with the equation C (ng/ml of plasma) $= 27.0(H/H_{IS})$. The mean coefficient of variation was 10%. Table II shows the day-to-day precision of one calibration curve over three days.

TABLE I

INTRA-DAY PRECISION OF CALIBRATION CURVES FOR FIVE DIFFERENT PLASMA SAMPLES

Amount added (ng/ml)	Amount found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)
1	1.03 ± 0.21	20.4
5	5.0 ± 0.50	10.0
10	10.0 ± 0.78	7.8
25	25.1 ± 1.32	5.3
50	49.8 ± 2.36	4.7
Mean		9.6

TABLE II

DAY-TO-DAY PRECISION OF CALIBRATION CURVE OVER THREE DAYS

Amount added (ng/ml)	Amount found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)
1	1.0 ± 0.18	18.0
5	5.1 ± 0.26	5.1
10	10.1 ± 0.25	2.5
25	25.2 ± 0.63	2.5
50	49.8 ± 1.04	2.1
Mean		6.0

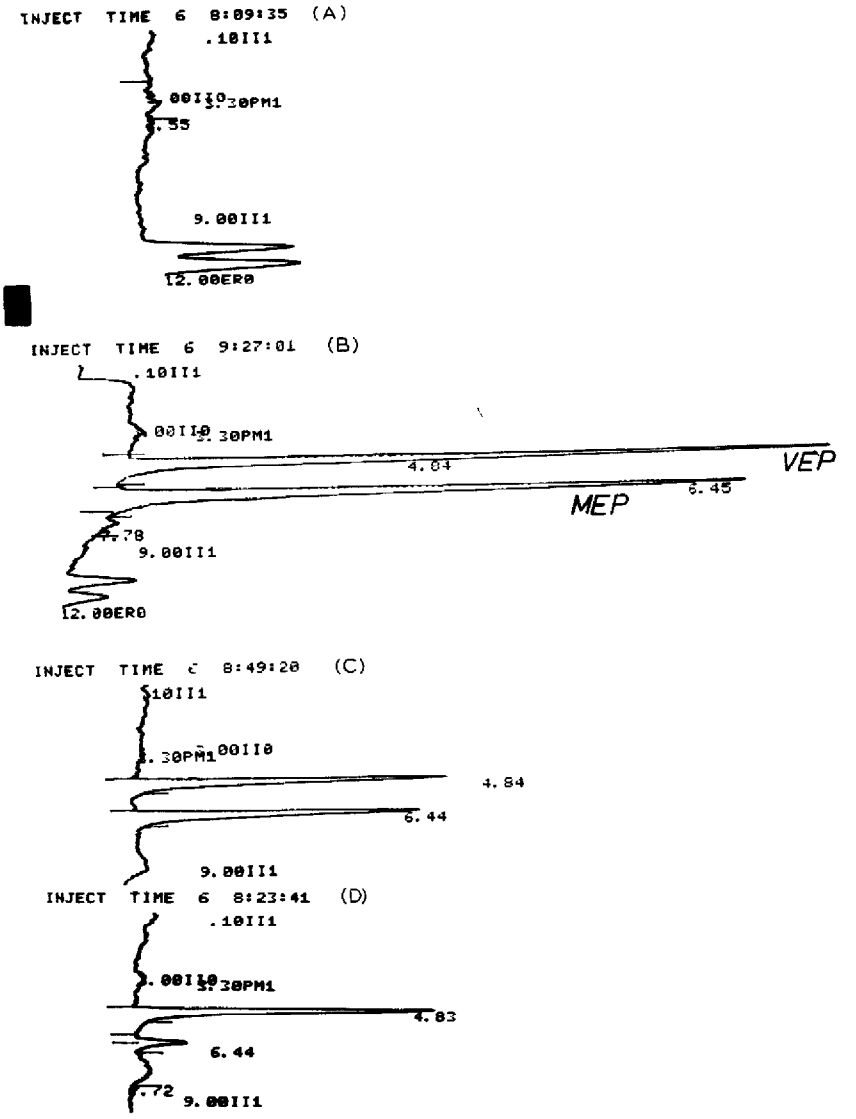

Fig. 1. Chromatograms of (A) a blank plasma sample, (B) a plasma sample spiked with MEP (25 ng/ml of plasma) and VEP (20 ng/ml of plasma) and (C,D) plasma samples taken from a healthy volunteer 2 h (C) or 4 h (D) after oral administration of 80 mg of MEP.

Chromatograms of an extracted blank plasma sample and an extracted calibration plasma sample at the calibration point 25 ng MEP per ml plasma are shown in Fig. 1. The apparent low recovery ($68 \pm 10\%$), which is shown in Table III, is caused principally by the extraction system; other systems give

TABLE III

RECOVERY OF MEP AS THE PEAK-HEIGHT RATIO OF PLASMA SAMPLE TO STANDARD SOLUTION

Amount added (ng/ml)	Recovery (mean ± S.D., $n=3$) (%)	Coefficient of variation (%)
1	76.3 ± 19.6	25.6
10	68.3 ± 1.5	2.2
20	67.0 ± 6.5	9.7
50	61.6 ± 2.5	4.1
Mean	68.3	10.4

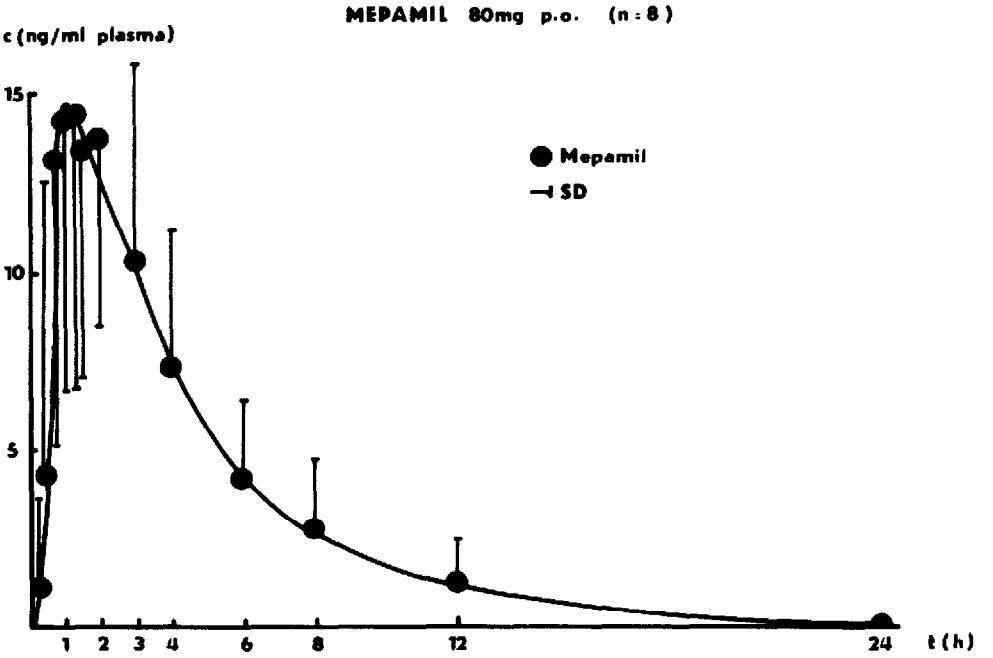

Fig. 2. Plasma concentration–time profile of MEP after oral administration of 80 mg of MEP.

greater recovery, but the analysis time increases (the plasma samples are contaminated). The recovery from plasma is expressed as the percentage ratio of the peak height of MEP in the plasma sample to that in the standard solution. The limit of detection (at a signal-to-noise ratio of 2) is 0.5 ng/ml of plasma. This explains the higher standard deviation at calibration point 1 ng/ml of plasma (Table I). MEP is stable in frozen plasma (−18.°C) for at least three months (99 ± 3%).

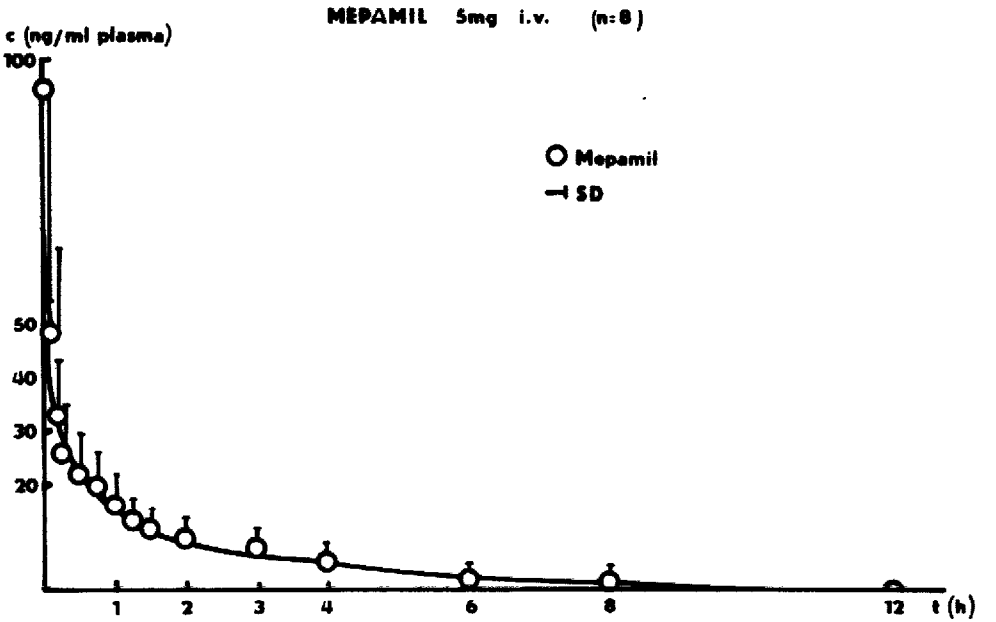

Fig. 3. Plasma concentration–time profile of MEP after intravenous administration of 5 mg of MEP.

With regard to further phases of clinical examination we tested interferences of other drugs. The interferences were evaluated by adding various drugs to plasma in amounts representative of therapeutic or higher concentrations in plasma. These were then treated as described above. The following drugs were tested: prazosin, metazosin, metoprolol, desacetyltrimepranol, furosemide, chlorthalidon and quinidine. None of these interfered with the analysis of MEP or VEP.

The method was applied in the first phase of clinical examination of MEP. The plasma levels were 1–50 ng/ml of plasma after a single oral administration of 80 mg of MEP (Fig. 2). The plasma levels after intravenous administration of 5 mg of MEP were 1–100 ng/ml of plasma (Fig. 3).

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