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Note

Estimation of concentrations of diltiazem in plasma using normal-phase column liquid chromatography with ultraviolet detection

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Several methods have been described for the measurement of diltiazem concentrations in plasma. These have mainly utilised gas chromatography [1—4] and column liquid chromatography (LC) [5—7]. Using gas chromatography, derivatisation reactions are required to resolve the parent compound, diltiazem, from the metabolite, desacetyldiltiazem, and to enhance the sensitivity of the procedure. The methods described for LC use reversed-phase chromatography and either acid back-extraction steps [5, 6] or the use of solid-phase extraction procedures [7].

The method described here uses a simple, single-stage extraction procedure followed by LC on a normal-phase column, with UV detection at 240 nm. The method is selective and sensitive with a limit of determination of 2.0 ng/ml.

EXPERIMENTAL

Materials

Ammonia, 18.1 *M* (Riedel de Haen, Hanover, F.R.G.); methanol, HPLC grade (Rathburn, Walkerburn, U.K.); hexane, diethyl ether and dichloromethane, all HPLC grade (Labscan, Dublin, Ireland); nitrogen, oxygen-free (Irish Industrial Gases, Athlone, Ireland); water, RO grade (Elga, High Wycombe, U.K.); disodium hydrogen phosphate and potassium dihydrogen phosphate, Analar grade (BDH, Poole, U.K.); diltiazem and internal standard (Elan Corporation, Athlone, Ireland).

The buffer solution used was prepared by mixing 20 ml of 0.067 *M* potassium dihydrogen phosphate with 80 ml of 0.067 *M* disodium hydrogen phosphate (pH 7.4).

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Chromatography

The apparatus comprised a Waters M45 pump and WISP autosampler, Shimadzu SPD6A UV detector (at 240 nm) and CR3A computing integrator. The column was a 25 cm × 4.6 mm I.D. Spherisorb S5W (Phase-Separations, Queensferry, U.K.). The mobile phase consisted of a mixture of ammonia (0.3 ml), methanol (30 ml), dichloromethane (30 ml) and hexane (360 ml) mixed in this order to allow proper mixing of all components. The mobile phase was delivered at a flow-rate of 3.0 ml/min, yielding a back-pressure of 96.5 bar.

Stock solutions

Diltiazem. A 1.0 mg/ml solution of diltiazem in methanol was prepared and from this, solutions of 1.0, 0.5, 0.2 and 0.1 μg/ml in methanol were made. A 100-μl aliquot of these was added to 1.0 ml of plasma to give amounts of 100, 50, 20 and 10 ng per ml of plasma.

Internal standard. (±)2,6-Dimethyl-4-(3-phenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid 3-[2-N-benzyl-N-methylamino]ethyl ester 5-methyl ester hydrochloride. Again a 1.0 mg/ml stock solution in methanol was prepared and from this a 1 μg/ml working solution was made. A 100-μl aliquot (100 ng) of this was added to 1.0 ml of specimen.

Extraction

To 1.0 ml of control or test plasma were added 100 μl of internal standard. To this were added 3.0 ml of extraction mixture (ether—hexane, 50:50) and the tubes were capped and mixed for 6 min on a standard laboratory shaker (approx. 30 rpm). After shaking, the tubes were centrifuged at 1500 *g* for 5 min. Following centrifugation, 2.0 ml of supernatant were transferred to plain glass tubes and evaporated to dryness at 50°C under a stream of nitrogen. Each extract was reconstituted in 150 μl of mobile phase and a 50-μl aliquot injected onto the column. Typical retention times were: internal standard, 2.9 min; diltiazem, 3.7 min; desacetyldiltiazem, 4.4 min (Fig. 1).

An unidentified peak elutes at 5.5 min and sufficient time should be left between injections to prevent interference with subsequent injections. However, if injections are made every 5 min this peak elutes with the unretained components of the following injection.

RESULTS

In order to calculate the concentrations of diltiazem in test specimens, a calibration line of peak-height ratio (diltiazem/internal standard) versus concentration of reference standards was drawn. The graph was linear over the standard range 0—400 ng/ml and passed through the origin ($r > 0.999$). From this graph the concentrations of drug in test specimens were calculated.

Using the method described herein the extraction of both diltiazem and internal standard was virtually complete (98%; coefficient of variation 7.2%) over the standard range used. The concentration—time profile obtained from one typical subject after receiving 120 mg of diltiazem orally, can be seen in Fig. 2.

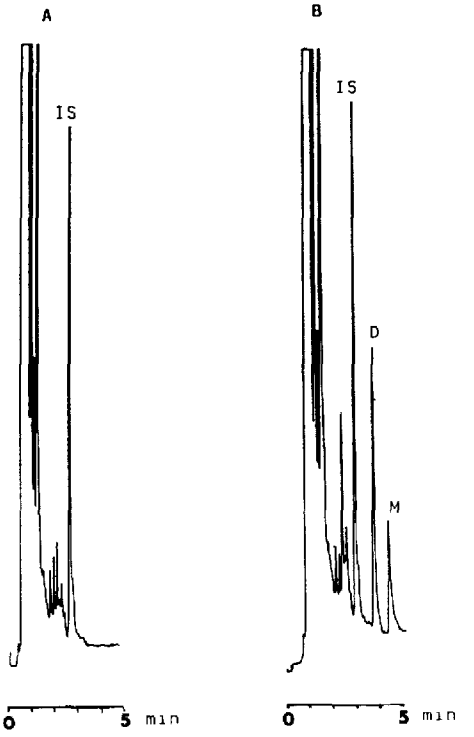

Fig. 1. (A) Chromatogram of a subject blank plasma extract containing internal standard. (B) Chromatogram of a subject plasma extract containing internal standard, 31 ng/ml diltiazem and 24 ng/ml desacetyldiltiazem. Peaks: IS = internal standard; D = diltiazem; M = desacetyldiltiazem.

Accuracy and precision

To assess the intra-assay variability of this method three concentrations (7.5, 15 and 75 ng/ml) of diltiazem in plasma were investigated and this showed a coefficient of variation of 6.0—7.4% (n = 6 at each concentration).

The inter-assay variability of the method was investigated at the same concentrations (n = 6) which showed a coefficient of variation of 7.2—8.0%.

DISCUSSION

Previous work [8] has demonstrated the usefulness of recycling the mobile phase. However, with the assay presented here there is a tendency for baseline noise and drift to occur if the mobile phase is recycled for more than 24 h. Our current policy is to prepare one new volume of mobile phase as described (approx. 420 ml) for every two batches of samples chromatographed (40—60 samples per batch). This method has allowed two batches (80—120 samples total) to be run daily by a single analyst.

With minor modification this method has been used for the estimation of diltiazem concentrations in a small number of urine specimens. To 1.0 ml of urine were added 1.0 ml of pH 7.4 phosphate buffer and 3.0 ml of hexane.

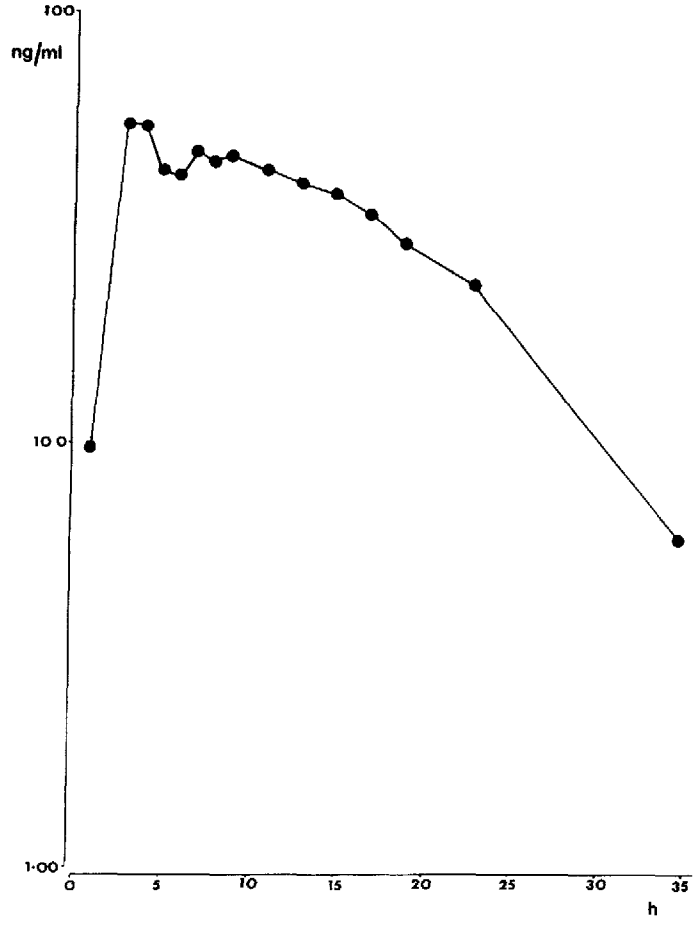

Fig. 2. Concentration—time profile from subject after a single oral dose of diltiazem, 120 mg.

The procedure was then followed as for that presented for plasma. It was necessary to adjust the pH of urine to neutral to maintain the high extraction yield which decreases significantly as the pH of the specimen becomes acidic (normal urinary pH range 4.6—8.2 [9]). Using hexane alone as extraction solvent, much cleaner chromatograms were obtained without reduction of the extraction efficiency. It should be further noted that although there is no change in the extraction efficiency in the range pH 7.4—14.0 it has been reported [6] that there is degradation of diltiazem at pH values greater than 8.5. A chromatogram of a urine extract can be seen in Fig. 3.

It can also be seen in Figs. 1 and 3 that desacetyldiltiazem is well resolved from the parent drug with no interfering peaks present in the specimen blanks.

CONCLUSION

The method presented herein provides a useful, simple and sensitive method for the estimation of diltiazem in large numbers of plasma specimens. Addi-

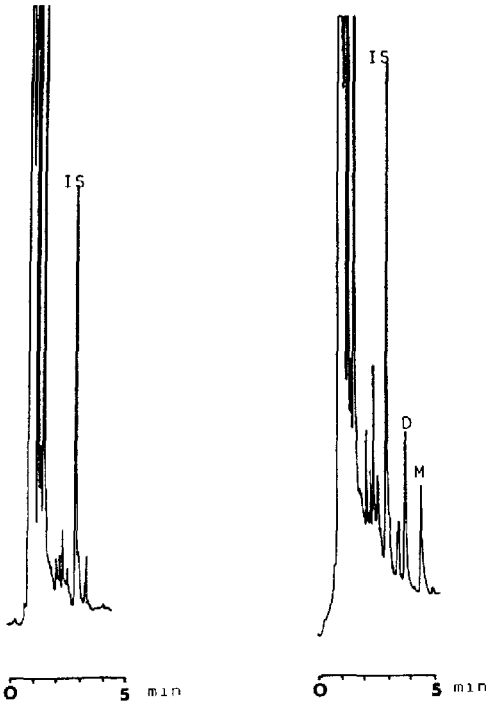

Fig. 3. (A) Chromatogram of a subject blank urine extract containing internal standard. (B) Chromatogram of a subject urine extract containing internal standard, 17 ng/ml diltiazem and 25 ng/ml desacetyldiltiazem. Peaks: IS = internal standard; D = diltiazem; M = desacetyldiltiazem.

tionally, the assay may be extended to allow the simultaneous quantification of diltiazem and desacetyldiltiazem in plasma and urine.

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