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SIMPLE, SENSITIVE AND SPECIFIC GAS CHROMATOGRAPHIC METHOD FOR THE QUANTIFICATION OF DILTIAZEM IN HUMAN BODY FLUIDS

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SUMMARY

A gas chromatographic method for the determination of the benzothiazepine diltiazem together with its major metabolite desacetyldiltiazem, is described. Silylation of the desacetyl derivative separates the metabolite from the parent drug on a 1% OV-17 column and cyclopam is used as an internal reference standard. The compounds are analysed by means of a nitrogen detector which allows the determination of 10 ng/ml of both compounds in plasma. The method has been used to determine both diltiazem and its desacetyl derivative in plasma obtained from healthy volunteers after oral doses of 60–210 mg of diltiazem.

INTRODUCTION

Diltiazem, *d*-3-acetoxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(*p*-methoxyphenyl-1,5-benzothiazepin-4(5H)-one, hydrochloride, has been shown to have potent coronary vasodilating activity without increasing the myocardial oxygen consumption in anaesthetized dogs. The compound also exhibits weak negative inotropic and chronotropic actions as well as a weak hypotensive activity[1–3]. Diltiazem is now used in Japan under the name Herbesser as a potent calcium antagonist antianginal drug[4–9].

The intensity and duration of the therapeutic and toxic effects of drugs are usually closely related to their biological availability and disposition. It is therefore important, in scheduling a rational therapy, to establish the pharmacokinetic profile of the drug by measuring its plasma levels after therapeutic doses.

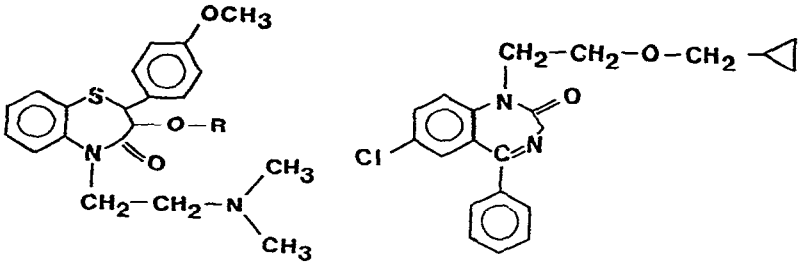
Data at present available on plasma diltiazem levels in man have been obtained by a method[10] which involves a preliminary thin-layer chromatographic (TLC) separation step followed by "spectrodensitometric" analysis at 237 nm. In view of the limited specificity and sensitivity of the absorptiometric method, we have developed a gas chromatographic procedure that is suitable for the assay of both diltiazem and its principle metabolite, desacetyldiltiazem, in plasma at therapeutic concentrations. The method has been used to determine plasma concentrations of these substances in preliminary studies on volunteers.

EXPERIMENTAL

Standard and reagents

Diltiazem hydrochloride (I) (DTZ) was supplied by Tanabe Seiyaku Co., Osaka, Japan. The desacetyl metabolite (II) (DAD) and cyclopam (IV) (CCP) were prepared in these laboratories; CCP was used as an internal reference standard. The structures of these compounds are given in Fig. 1.

The reagents used were *n*-hexane and ethyl acetate (analytical reagent grade, Merck, Darmstadt, G.F.R.) and N,O-bis(trimethylsilyl)acetamide (BSA; Pierce, Rockford, Ill., U.S.A.).



R = $COCH_3$ (I) CCP (IV)

R = H (II)

R = $Si(CH_3)_3$ (III)

Fig. 1. Structures of diltiazem (I), desacetyldiltiazem [before (II) and after (III) silylation] and cyclopam (IV).

Apparatus

A Perkin-Elmer 910 gas chromatograph equipped with a nitrogen–phosphorus detector (NPD) was used with a glass column, 2 m long × 4 mm I.D., packed with Chromosorb W (80–100 mesh) coated with 1% OV-17 (Applied Science Labs., State College, Pa., U.S.A.). The column was initially conditioned for 1 h at 300° with a nitrogen flow-rate of 40 ml/min, then for 4 h at 300° with no nitrogen flow, and finally for 24 h at 270° with a nitrogen flow-rate of 40 ml/min.

Operating conditions

The column was operated at 270° with injection port and detector temperatures of 290° using helium as the carrier gas at a flow-rate of 50 ml/min. The rubidium bead in the NPD detector was heated to about 600° (helipot at 620°) and the detector polarity was set at −180 V using a hydrogen flow-rate of 2 ml/min and an air flow-rate of 100 ml/min.

Extraction procedure

The extraction procedure is shown schematically in Fig. 2. An ethanolic solution (40 μl containing 10 ng/ml) of CCP was evaporated to dryness under nitrogen in

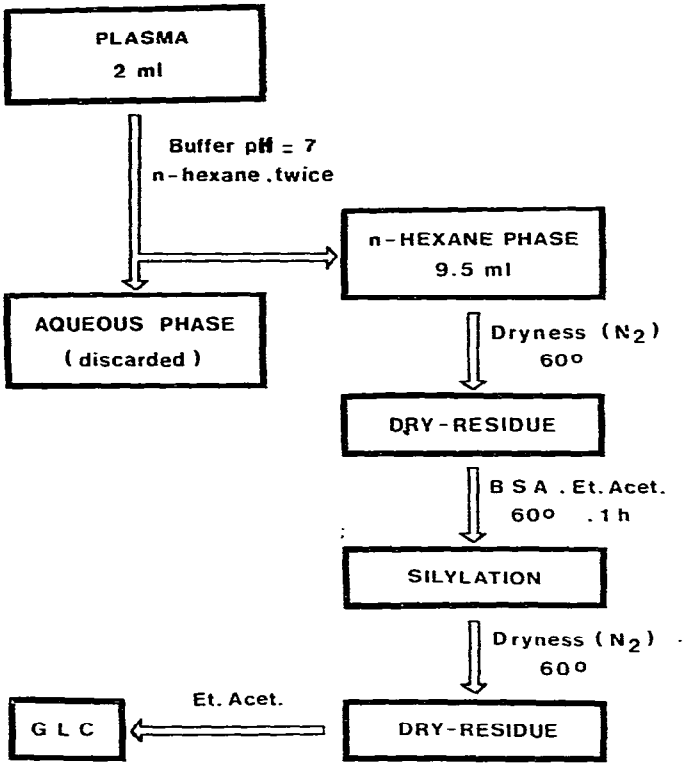


Fig. 2. Extraction procedure for diltiazem and desacetyldiltiazem.

a tapered tube. The plasma sample (2 ml) and 1 ml of phosphate buffer (0.2 *M*, pH 7.0) were then added to the tube, and this mixture was extracted with *n*-hexane, separated after centrifugation (5 min) and the extraction repeated with a second volume of *n*-hexane. The combined *n*-hexane extracts were then evaporated to dryness under nitrogen at 60°. Ethyl acetate (100 μl) and BSA (20 μl) were added to the dried extract, which was then heated for 60 min at 60°. The mixture was again evaporated to dryness under nitrogen and excess of BSA reagent removed by evaporation with a second volume of ethyl acetate (50 μl). Finally, 20 μl of ethyl acetate were added to the residue and 1 μl was injected into the column.

RESULTS

A typical chromatogram is compared with that obtained from a blank plasma sample in Fig. 3. It can be seen that the peaks corresponding to DTZ, DAD and CCP are well resolved and that there is no interference from endogenous substances present in plasma.

The retention times were 3.80 min for DAD, 5.10 min for CCP and 7.20 min for DTZ, and the heights equivalent to a theoretical plate (HETP) were 0.81, 0.72 and 0.72 mm for DAD, CCP and DTZ respectively. The resolution factors were 1.54 for DAD–CCP and 1.65 for CCP-DTZ.

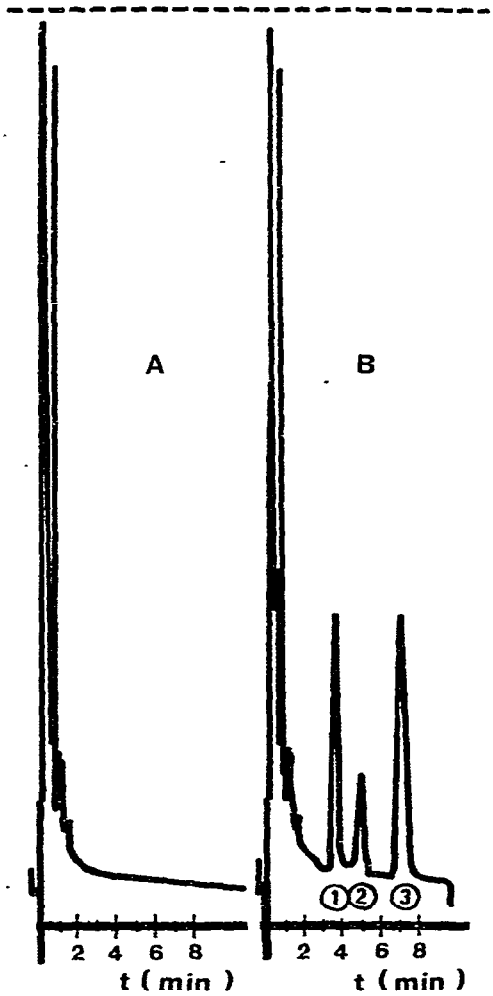


Fig. 3. Gas chromatograms of plasma extracts. A = Blank plasma control sample; B = plasma sample containing DAD (1), cyclopam (2) and DTZ (3).

The minimum detectable amounts in plasma were 10 ng/ml for DTZ and DAD and the absolute sensitivities of the NPD detector were 0.681, 0.482 and 0.543 Cb per gram of nitrogen for DAD, CCP and DTZ, respectively.

The calibration graphs presented in Fig. 4 were prepared using the peak-height ratio method. Each point was obtained in triplicate. A linear response was obtained for both compounds over a 100-fold change in concentration. A second calibration graph was prepared over a smaller concentration range (10–200 ng/ml in plasma) and the data, together with standard deviations, recoveries and accuracies, are given in Table I. Correlation coefficients of 0.998 and 0.997 were obtained for DAD and DTZ, respectively, over the entire concentration range studied.

Plasma samples were obtained from volunteers who received single and repeated oral doses of diltiazem. Two typical series of chromatograms are presented

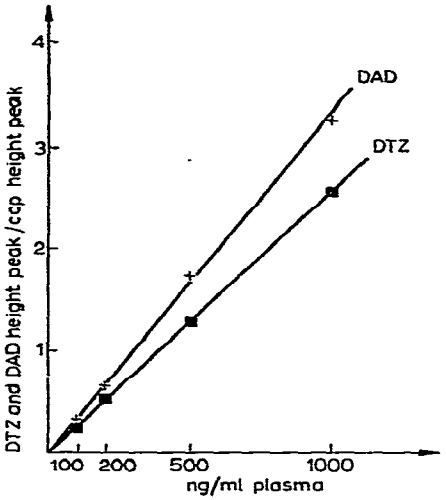


Fig. 4. Calibration graph for quantitative analysis of DTZ and DAD.

TABLE I

CHARACTERISTICS OF THE ANALYSIS OF DTZ AND DAD IN PLASMA AT DIFFERENT CONCENTRATIONS

Internal standard cyclopam (CCP); 200 ng/ml in plasma.

DTZ				*DAD*			
Amount (ng/ml in plasma)	*DTZ/CCP peak-height ratio*	*Standard deviation*	*Accuracy (S.E., %)*	*Amount (ng/ml in plasma)*	*DAD/CCP peak-height ratio*	*Standard deviation*	*Accuracy (S.E., %)*
10	0.030	± 0.0005	1.9	10	0.031	± 0.001	4.8
50	0.127	± 0.003	1.4	50	0.158	± 0.021	0.3
100	0.260	± 0.019	4.3	100	0.323	± 0.041	6.0
200	0.530	± 0.032	3.5	200	0.594	± 0.052	1.7
500	1.295	± 0.024	1.1	500	1.710	± 0.028	1.2
1000	2.620	± 0.060	1.6	1000	3.189	± 0.189	4.2
		Recovery: 70%				Recovery: 55%	
		Regression coefficient: 0.997				Regression coefficient: 0.998	

in Fig. 5. The first were obtained from plasma samples taken from a subject who had received a single, initial dose of 180 mg.

The second series was obtained from samples taken from the same subject at similar intervals after another 180 mg dose given after a 7-day period of regular treatment with 60 mg of diltiazem three times daily. No interference was apparent in either series of chromatograms.

Analyses of plasma samples taken from other subjects showed that maximum plasma diltiazem levels ranged from 50 to 500 ng/ml after doses of 60–210 mg. Plasma levels of the order of 10 ng/ml could be detected 12–24 h after a dose of 60 mg. The compound appeared to have an apparent half-life of 4–6 h. A detailed pharmacokinetic analysis will be published later.

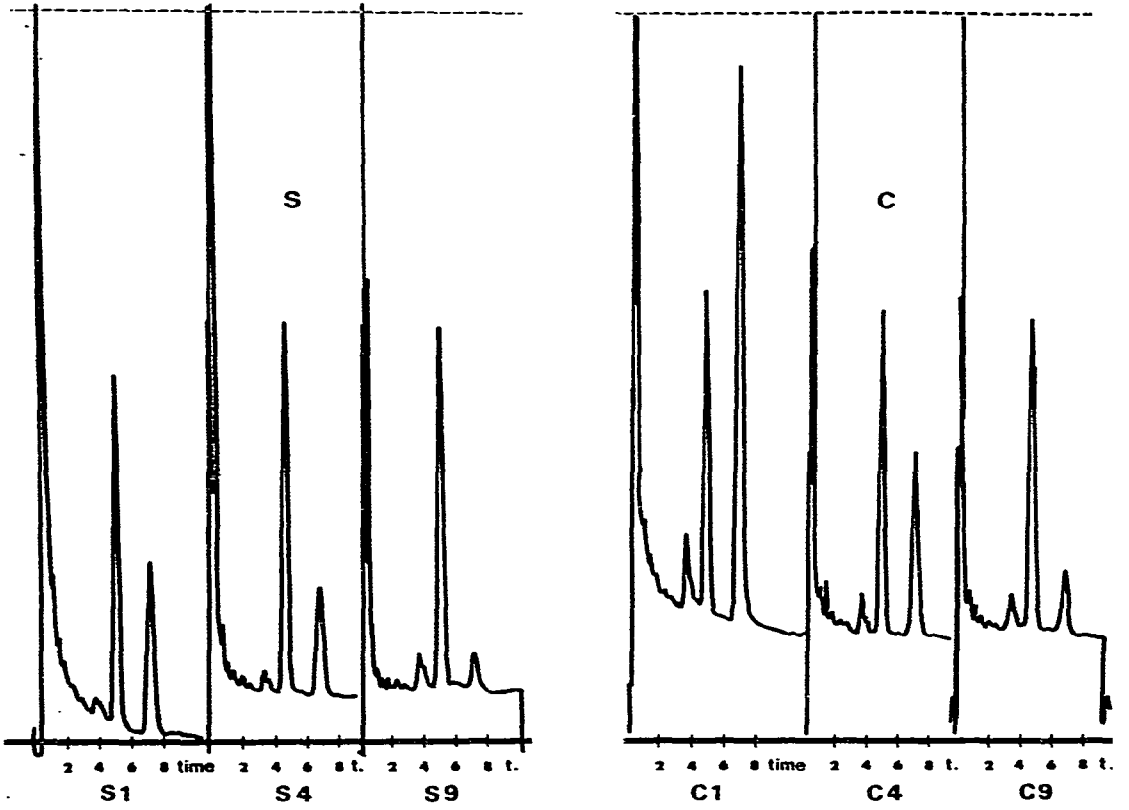


Fig. 5. Gas chromatograms of plasma extracts obtained 1, 4 and 9 h after 180 mg of DTZ administered orally as single dose (S) and after a repeated treatment (C).

Plasma DTZ and DAD levels obtained in the same patients after single and repeated treatments with DTZ are shown in Fig. 6. The plasma levels of DAD were about 10% of those for DTZ.

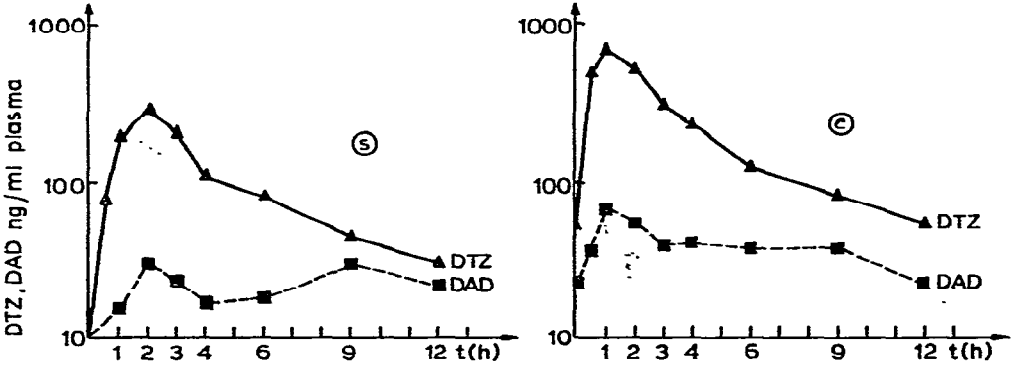


Fig. 6. DTZ and DAD plasma concentrations in a healthy volunteer after 180 mg of DTZ administered orally as single dose (s) and after a repeated treatment (c).

DISCUSSION

Our initial studies with diltiazem established that both DTZ and DAD could be detected by means of a flame-ionization detector. However, because of a large solvent tail, the minimum amount of DTZ that could be detected was 10 ng, corresponding to 200 ng/ml in plasma.

Recent work on NPD[11,12] has established that this detector is particularly useful for the determination of nitrogen-containing drugs in biological fluids. In this study, only the method developed with the NPD detector was sufficiently sensitive to measure DTZ in human plasma utilizing small blood samples. In our hands, the response of the Perkin-Elmer nitrogen detector remained constant over a period of more than 200 working hours, which made it possible to develop a method with a high degree of accuracy having a linear response over at least a 100-fold range of concentration. Initial studies were carried out with a column containing 3% OV-17 on which DTZ and DAD were well resolved, but the peaks were broad and their retention time was about 20 min. However, when the amount of stationary phase was reduced to 1%, the peaks corresponding to DTZ and DAD could no longer be completely resolved. Experiments with the silyl derivative of DAD established that this could be separated from DTZ and, as can be seen in Fig. 3, DTZ and DAD-silyl (III) are well resolved in a short time on the 1% column. As silylation occurs at the 3-hydroxy position, no silyl derivative of DTZ is formed. The internal standard reference (CCP) is also unaltered during silylation.

Analysis of fresh plasma by this method showed that interfering substances are absent, and we have been able to determine DTZ at a concentration of 10 ng/ml in plasma samples taken from volunteers; the metabolite DAD was also detected and it was possible to measure it at concentrations of 10–60 ng/ml in plasma. Old plasma samples (3–4 weeks) often contained a substance with a peak retention time close to that of DAD. When this substance was present in small amounts, as in Fig. 5, the accuracy of the analysis was unaffected. However, the interference was sometimes such as to make analysis impossible. It is therefore essential to analyse plasma samples immediately for an accurate determination of DAD.

In conclusion, the procedure described here provides a rapid method for the determination of DTZ and DAD in plasma samples because of its sensitivity, specificity and simplicity. This procedure is superior to the earlier absorptiometric method and is suitable for monitoring plasma levels during both chronic treatment and single-dose pharmacokinetic studies.

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