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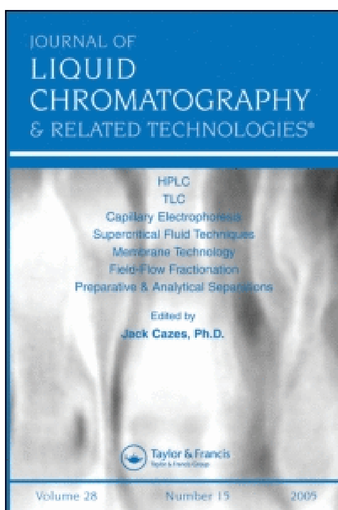

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An HPLC Method for the Determination of Diltiazem and Desacetyldiltiazem in Human Plasma

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AN HPLC METHOD FOR THE DETERMINATION OF DILTIAZEM AND DESACETYLDILTIAZEM IN HUMAN PLASMA

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ABSTRACT

A high performance liquid chromatographic method is presented for the determination of diltiazem and its metabolite desacetyldiltiazem in human plasma. Diltiazem and desacetyldiltiazem are extracted from plasma basified with 0.5M dibasic sodium phosphate (pH 7.4) using 1% 2-propanol in n-hexane containing diazepam as an internal standard. A reversed phase cyanopropylsilane column was used with a mobile phase of 45% acetonitrile and 55% 0.05M acetate buffer (pH 4.0). The minimum detectable limit was 2ng/ml of plasma. The effect of the pH, molarity, and percent acetonitrile of the mobile phase on the capacity factor was studied. Possible interferences from other drugs administered concurrently are presented.

INTRODUCTION

Diltiazem, cis-(+)-3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one

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hydrochloride is a calcium channel blocking agent useful in the treatment of vasospastic angina and chronic stable angina (1).

Diltiazem appears to be well tolerated orally, and is extensively metabolized by the liver to the active metabolite desacetyldiltiazem and four inactive metabolites (2). The nature and incidence of adverse side effects resulting from the use of diltiazem is relatively minor (3). But, concomitant administration of diltiazem with other commonly prescribed drugs could be dangerous and contraindicated in some situations (4). Due to the wide range of plasma levels and concurrent administration of other commonly prescribed drugs, monitoring patient plasma levels is helpful to the clinician to minimize side effects and maximize therapeutic response.

Methods to determine diltiazem include gas chromatographic procedures which have time consuming extraction processes or require silylation of the metabolites for detection (5,6). High performance liquid chromatographic methods are available that either lack sensitivity (7,8), or are not applicable to human biological fluids (9).

This paper describes the separation and quantitation of diltiazem and its active metabolite desacetyldiltiazem in human plasma by reversed phase HPLC using a cyanopropylsilane column after a single extraction step.

The method is sensitive and applicable to the monitoring of plasma levels even in the presence of drugs which may be prescribed concurrently.

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MATERIALS AND METHODS

Instrumentation

A Hewlett Packard Model 1090 liquid chromatograph was equipped with an automatic injector, a Dupont cyanopropylsilane column (15 cm long and 4.6 mm i.d.), and a variable wavelength diode array detector set at 239 nm. The signal was recorded on a Hewlett Packard Model 3392A integrator. The degassed mobile phase was pumped through the column at 1.5ml/min and the column compartment was maintained at 40°C.

Chemicals and Reagents

Sodium hydroxide, dibasic sodium phosphate, sodium acetate, and n-hexane were reagent grade. 2-Propanol was histological grade, and acetonitrile and methanol were HPLC grade. Diltiazem hydrochloride and desacetyldiltiazem were supplied by Marion Laboratories, Inc. Diazepam was supplied by Roche Laboratories, Division of Hoffmann-LaRoche, Inc.

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Drug and Metabolite Solutions

Separate solutions of diltiazem and desacetyldiltiazem were made containing 5mg/100ml of methanol. Working dilutions of 1 and 10ug/ml methanol were prepared from the stock solutions.

Internal Standard Solutions

A stock solution of diazepam was prepared at 5mg/100ml methanol. A working dilution of 5ug/ml methanol was prepared from the stock solution. The extraction solution consisted of a 2.5ml aliquot of the working solution in 1000ml n-hexane containing 1% 2-propanol (v/v).

Mobile Phase

Sodium acetate solution, 0.05M was prepared in deionized distilled water and the pH was adjusted to 4.0. The mobile phase consisted of 55% buffer and 45% acetonitrile.

The effect on the capacity factor (k') of changes in the mobile phase pH, molarity, and percent acetonitrile were studied.

Preparation of Plasma Standards

To 1.0ml of plasma in a 15ml screw capped centrifuge tube, aliquots of working drug and working metabolite solutions were added to simulate drug and metabolite concentrations (40-280ng/ml of plasma). The spiked plasma was basified with 0.5ml Na_2HPO_4 buffer (0.5M, pH 7.4), and 10ml of the extraction solution containing the internal standard was added. The tubes were vortexed for 20 seconds and centrifuged for 10 minutes at 900xg. The organic phase was transferred to an open top centrifuge tube and evaporated to dryness at room temperature under a gentle stream of nitrogen. The residue was dissolved in 100ul of acetonitrile and transferred to a glass HPLC vial. A 20ul aliquot was injected onto the column.

Quantitation

Standard curves were constructed utilizing three replicates at each concentration. The peak heights were measured, and the ratios (drug/internal standard and metabolite/internal standard) were calculated and plotted against the concentration of drug added in nanograms per milliliter of plasma.

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Recovery

Plasma samples containing a known concentration of diltiazem and desacetyldiltiazem were carried through the analysis. An equivalent concentration of drug and metabolite was added to 10ml of extraction solution, vortexed, evaporated to dryness, and reconstituted in acetonitrile for injection into the chromatograph. The peak heights of the drug, metabolite, and internal standard in the plasma samples were measured for comparison against the samples without extraction to estimate recovery.

Interferences

The possible interference from normal plasma constituents has been studied by the analysis of drug free plasma samples. The interference of other drugs was tested by direct injection of aqueous or methanolic drug solutions under the same chromatographic conditions.

RESULTS AND DISCUSSION

The chromatographic conditions were chosen to obtain good separation of diltiazem, desacetyldiltiazem and diazepam, minimum interference from endogenous plasma constituents, and reasonable retention times. Sufficient sensitivity for the detection of diltiazem and desacetyldiltiazem was achieved using ultraviolet detection at 239nm. The choice of wavelength was based on a spectrum scan. The limit of detection for both the drug and its metabolite was 2ng/ml of plasma based on a peak height twice the baseline noise.

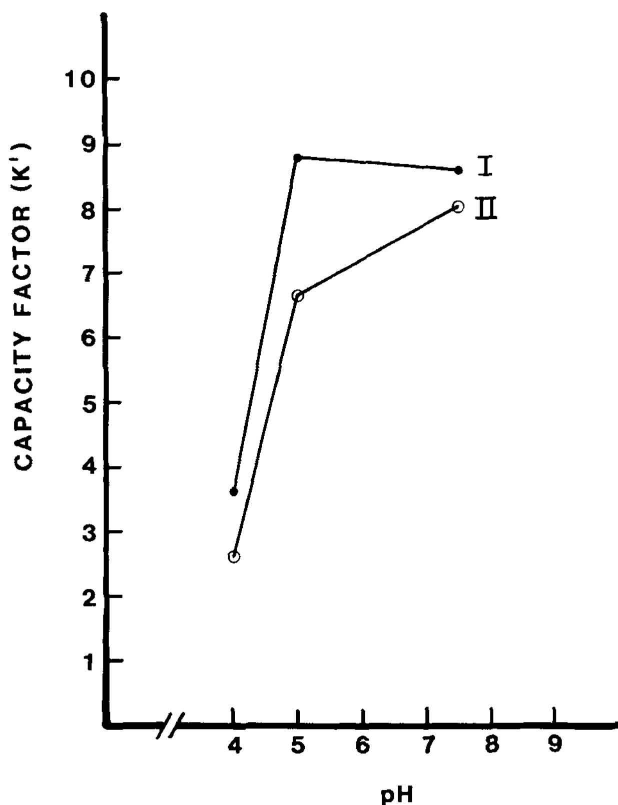

Figure 1. Effect of pH of the mobile phase buffer on capacity factor (k') at 0.05M and 45% acetonitrile.

Key: I, diltiazem; II, desacetyldiltiazem

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To obtain good chromatographic resolution of diltiazem and desacetyldiltiazem, the pH, molarity, and percent acetonitrile in the mobile phase were varied independently to determine their effects on the capacity factor (k'). As the mobile phase pH was increased above 5, the capacity factor decreased and peak broadening occurred (Figure 1). Changes in mobile phase molarity

showed a slight effect on the capacity factor (Figure 2), while the percent of acetonitrile in the mobile phase had the most dramatic effect (Figure 3). A mobile phase consisting of 45% acetonitrile and 55% acetate buffer (0.05M, pH 4) (v/v) afforded good chromatographic resolution using a cyanopropylsilane column in a reversed phase mode.

Diazepam was chosen as the internal standard since it extracts with the drug and metabolite, it resolves well from the drug, metabolite, and endogenous plasma constituents, and it can be determined using the same chromatographic conditions.

Diltiazem and desacetyldiltiazem can be readily extracted from basified plasma at pH 7.4 using hexane containing 2-propanol (99:1, v/v) in a single extraction step. The choice of extraction conditions were based on obtaining a high recovery of drug and metabolite with minimum interference from plasma constituents. The percent recovery from samples containing 200ng/ml plasma for the method described was 89% for diltiazem, and 84% for desacetyldiltiazem. Typical chromatograms from plasma extracts are found in Figure 4.

The ratio of peak height of diltiazem or its metabolite to the peak height of the internal standard was plotted versus drug concentration added to plasma. Statistical analysis of the data by linear regression indicated good reproducibility and linearity in the range of 40.0-280.0ng/ml plasma (Table 1). The use of an internal standard in the extraction solution allowed for simple sample processing without correction for extraction recovery.

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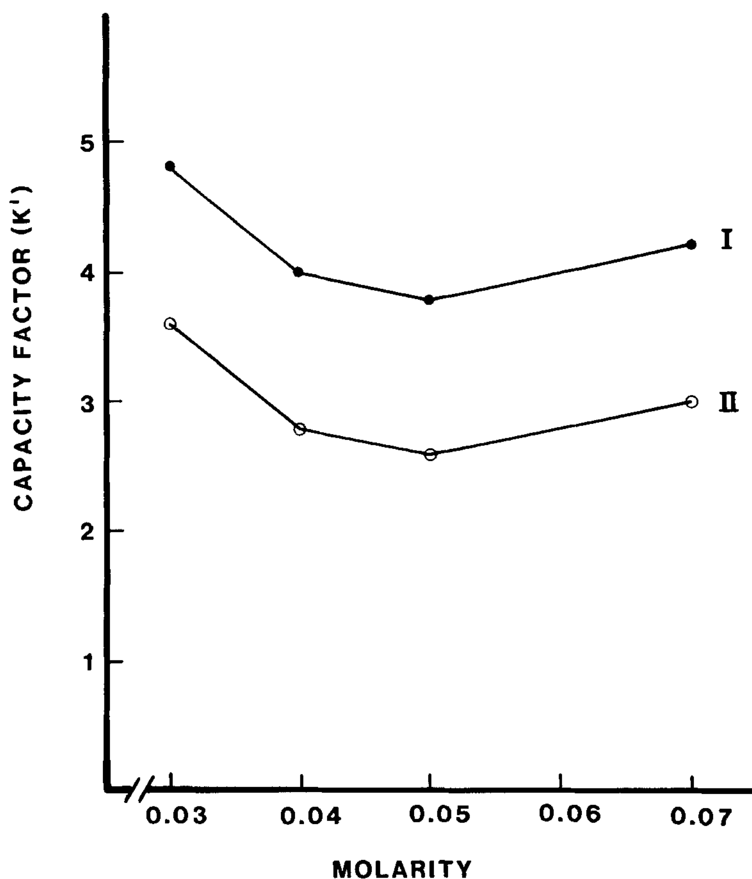

Figure 2. Effect of molarity of the mobile phase buffer on capacity factor (k') at pH 4.0 and 45% acetonitrile.

Key: I, diltiazem; II, desacetyldiltiazem

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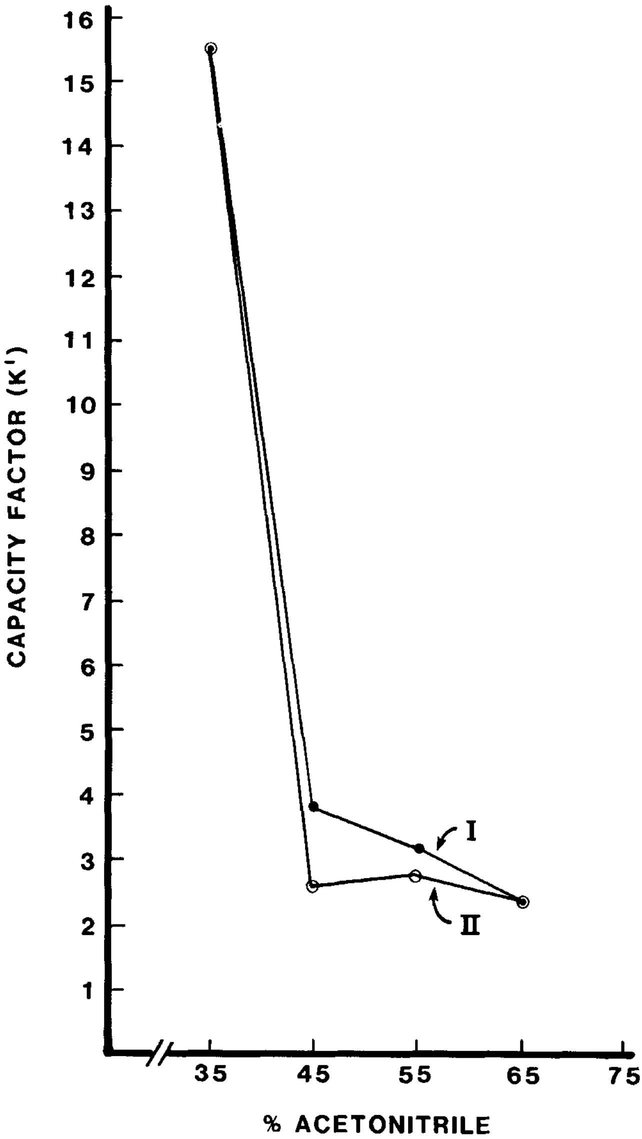

Figure 3. Effect of percent acetonitrile in the mobile phase on capacity factor (k') at 0.05M and pH 4.0.

Key: I, diltiazem; II, desacetyldiltiazem

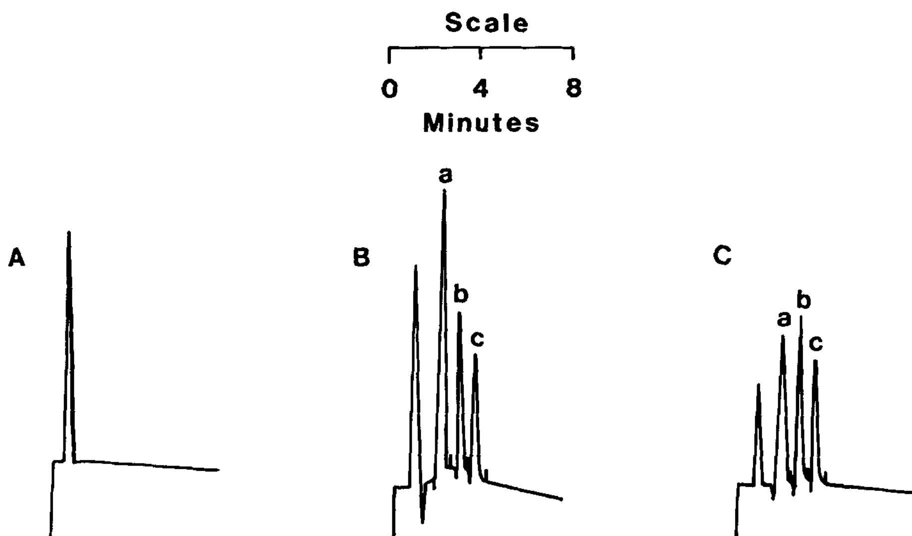

Figure 4. Typical chromatograms of plasma extracts containing diazepam (a), desacetyldiltiazem (b) and diltiazem (c).

Key: A, drug free plasma; B, 120ng each of drug and metabolite/ml plasma; C, 240ng each of drug and metabolite/ml plasma

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TABLE 1

Linearity and Precision of the Diltiazem and Desacetyldiltiazem Plasma Assay

Concentration Added (ng/ml plasma)	Concentration Found(ng/ml plasma)*	
	Diltiazem	Desacetyldiltiazem
40.0	47.0 ± 1.6	39.2 ± 1.0
80.0	90.5 ± 1.7	80.2 ± 3.9
120.0	122.4 ± 0.3	127.7 ± 2.1
160.0	161.5 ± 2.5	162.5 ± 2.5
200.0	199.1 ± 4.1	200.2 ± 3.5
240.0	237.8 ± 2.3	236.5 ± 4.2
280.0	279.1 ± 6.0	287.5 ± 7.2
Correlation Coefficient	0.9996	0.9989
Slope	0.9532	1.0089
Y-Intercept	9.9714	0.5429
N	3	3

* Mean ± Standard Deviation

TABLE 2

Drugs Tested for Possible Interference
With Diltiazem and Desacetyldiltiazem Using UV Detection
at 239 nm

Drug	Retention Time (min)
Furosemide	Solvent Front
Chlorthalidone	Solvent Front
Methyldopa	Solvent Front
Atenolol	Solvent Front
Pentoxifylline	Solvent Front
Hydralazine	Solvent Front
Procainamide	2.29
Labetalol	2.34
Diazepam (IS)	2.50
Warfarin	2.54
Metoprolol	2.55
Pindolol	2.61
Isosorbide Dinitrate	2.64
Dipyridamole	3.00
Disopyramide	3.39
Propranolol	3.82
Desacetyldiltiazem	3.82
Quinidine	3.87
Chlorothiazide	4.25
Diltiazem	4.90
Captopril	7.17

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The retention times of certain drugs which might be administered concurrently with diltiazem are presented in Table 2. The results indicate no interference with the parent drug, but some compounds may co-elute with the metabolite or internal standard.

CONCLUSIONS

The HPLC method described in this paper uses a cyanopropylsilane column under isocratic elution conditions and offers

excellent separation of diltiazem, its metabolite and the internal standard, with a relatively short analysis time, and good sensitivity.

The method is simple and rapid as it requires only a single extraction step prior to injection. The method is linear and reproducible and can be utilized for routine patient monitoring or for pharmacokinetic studies.

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