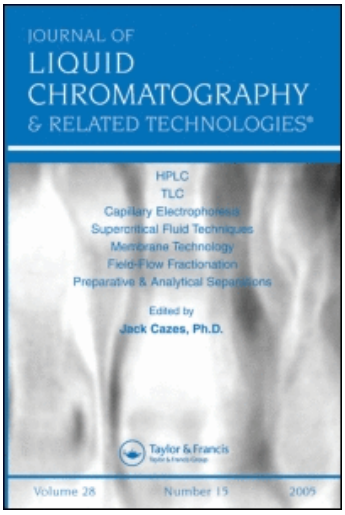

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DILTIAZEM: A SENSITIVE HPLC ASSAY AND APPLICATION TO PHARMACOKINETIC STUDY

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ABSTRACT

A sensitive high-performance liquid chromatographic (HPLC) method for the determination of diltiazem in human plasma has been developed. Propranolol was used as the internal standard. The assay uses a reversed phase C18 microbore column (2 mm I.D. x 10 cm) packed with 5 μm ODS Hypersil. The chromatographic was achieved by using an isocratic mobile phase comprising acetonitrile-aqueous phosphate buffer (40:60, v/v) containing 40 mM sodium dodecyl sulphate and 3 mM tetrabutylammonium bromide adjusted to pH 2. The mobile phase was pumped a flow rate of 0.5 ml/min. The eluant was monitored by a UV detector operating at 240 nm. The assay was based on an organic extraction with tert-butylmethyl ether and then back-extracted into a small volume of acidic aqueous solution before injection onto the HPLC column. With this procedure coefficients of variation were less than 10%. The detection limit was 1 ng/ml of plasma. The method is sensitive, selective and allows for routine analysis in the pharmacokinetic studies.

INTRODUCTION

Diltiazem is a benzothiazepine derivative calcium antagonist. It is widely used in the treatment of various cardiovascular disorders such as angina pectoris (1). Several high-performance liquid chromatographic (HPLC) methods have been developed (2-5). These methods allow for the simultaneous determination of

diltiazem and its metabolites in biological fluids. However, these assays have detection limits of 10 ng/ml in plasma and 100 ng/ml in urine, so they are not suitable for precise pharmacokinetic studies after single doses. The present report describes a sensitive HPLC method for simultaneous quantitation of diltiazem and its metabolites in human plasma. The applicability of this procedure is demonstrated by the analysis of plasma samples from subjects receiving a single oral dose of diltiazem in a bioavailability study.

MATERIALS AND METHODS

Reagents and Chemicals

Diltiazem hydrochloride was kindly supplied by Douglas Pharmaceuticals Ltd., Auckland, New Zealand. Propranolol hydrochloride was obtained from Pacific Pharmaceutics Ltd (Auckland, New Zealand). Sodium dodecyl sulphate (SDS), orthophosphoric acid, HPLC-grade acetonitrile and tert-butylmethyl ether were purchased from BDH Chemicals Ltd (Poole, England). Tetrabutylammonium bromide was purchased from Sigma Chemical Co (St Louis, MO, USA). All chemicals used were analytical grade. Glassware was cleaned and silanized with 0.05% Aquasil® (Pierce Chemical Co., Rockford, IL, USA) before use. Water was double glass distilled and MilliQ® filtered.

Standard Solutions and Internal Standard

A stock solution containing 1 mg/ml diltiazem was prepared in methanol. This solution was found to be stable for at least 1 week at -20°C. Plasma standard solutions of diltiazem for the calibration curves were prepared by proper dilution of the stock solutions with drug-free plasma so that concentrations of 1, 5, 10, 20 and 50, 100, 200 and 500 ng/ml were obtained. The internal standard solution of propranolol hydrochloride (3 μg/ml) was prepared in HPLC-grade water. This solution was prepared fresh every second day of analysis and stored at 4°C until required.

Sample Preparation

To 2 ml of plasma in a silanized centrifuge tube, 100 μl of internal standard solution (3 μg/ml propranolol hydrochloride) was added. The contents were then

shaken with 6 ml tert-butylmethyl ether for 15 minutes. The samples were centrifuged for 15 minutes at 1500 g to separate the phases. The organic layer was transferred to a clean tapered glass centrifuge tube containing 100 µl of 0.05 M sulphuric acid. The mixture was shaken for 15 minutes and centrifuged (1500 g, 4°C) for 10 minutes. The organic layer was aspirated and discarded. The aqueous extract was transferred to the autosampler plastic vials and 50 µl was injected onto the HPLC column. The samples were stored at 4°C until injection.

Chromatographic Conditions

The HPLC system consisted of a LKB 2150 pump (LKB, Stockholm, Sweden) connected to a Waters 712 autoinjector (Milford, MA, USA) with samples stored at 4°C until injection. The detector used was a Linear UVis 200 spectrophotometer (Linear Instruments Corp., Nevada, USA) operated at 240 nm using a setting of 0.01 a.u.f.s. The chromatographic response was recorded by a Shimadzu R3A integrator (Shimadzu, Kyoto, Japan). A microbore HPLC column (2 mm I.D. x 10 cm) packed with 5 µm ODS Hypersil (Shandon, London, UK) was used. The column efficiency was over 4000 plates per 10 cm. Analysis of the samples of diltiazem was performed using a mobile phase consisting of an acetonitrile-aqueous phosphate buffer (10 mM Na_2HPO_4) mixture (40:60, v/v) containing 40 mM sodium dodecyl sulphate (SDS) and 3 mM tetrabutylammonium bromide (TBA) and adjusted to pH 2 with orthophosphoric acid. The flow rate of the mobile phase was 0.5 ml/min (back pressure approximately 110 bars). Chromatographic separations were performed at room temperature.

Recovery

The assay recovery of diltiazem from plasma was determined at 10 and 100 ng/ml. Absolute recovery was calculated by comparing the peak heights from 6 extracted plasma samples with those obtained by direct injection of the pure drug standard of diltiazem. The absolute recovery of the internal standard (propranolol) was assessed using the same procedure.

Calibration Curve

Standards corresponding to 0, 1, 5, 10, 20, 50, 100, 200 and 500 ng/ml of diltiazem were prepared in plasma. The sample analysis was performed as

described above and standard curves were run daily. Quantitation is based on peak height ratios (diltiazem/internal standard). An unweighted least squared regression was fitted to each individual calibration curve.

Clinical Study: Bioavailability of Diltiazem Tablet

Eighteen healthy male volunteers between the age of 18 and 25 were recruited for the randomized two way crossover study. The study was approved by the local ethical committee. Each Subject gave his signed informed consent. The study was designed to determine the relative bioavailability of a new formulation of diltiazem 60 mg tablets. (Douglas Pharmaceuticals Ltd, Auckland, New Zealand), thereafter noted *Dil-DP*. This was compared to the currently marketed formulation, 60 mg Cardizem® tablets (ICI Australia Operations Pty Ltd, Melbourne, Australia), thereafter noted *Cardizem*. A single oral dose of diltiazem 180 mg (3 tablets) of either formulation was given to the subjects in the morning. Blood samples were withdrawn at intervals following drug administration up to 28 hours. Plasma was separated by centrifuged (400 g for 10 minutes) and kept frozen at -70°C until analysis.

Statistical Analysis

Results given are mean ± S.D. Student *t-test* was used throughout the study unless otherwise stated.

RESULTS AND DISCUSSION

The liquid chromatographic separation of diltiazem, its known metabolites and the internal standard (propranolol) from the endogenous plasma peaks was achieved by using a reversed phase C18 microbore column with an acetonitrile-aqueous phosphate buffer mixture (40:60, v/v) containing 40 mM SDS and 3 mM TBA adjusted to pH 2. Composition of the mobile phase was obtained from the mathematical model to the chromatographic behaviour previously described (6). The present HPLC assay developed is selective for diltiazem in the presence of its four metabolites and the internal standard (Figure 1). The peaks of diltiazem, its metabolites and the internal standard were well resolved. The retention times for

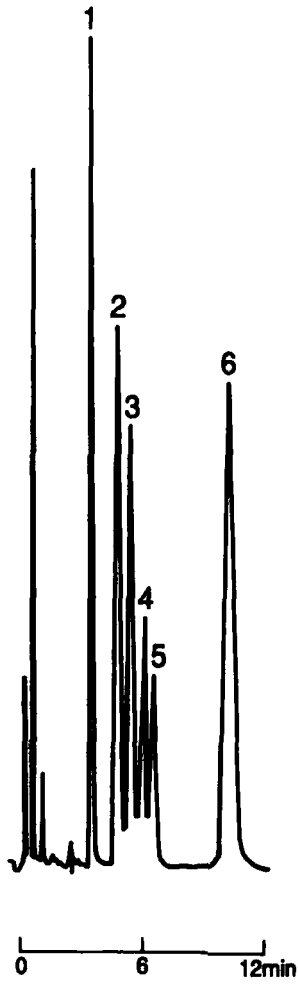

FIGURE 1

Chromatogram showing separation of diltiazem and its metabolites and the internal standard (propranolol). Drugs and metabolites were prepared in water.

Peaks: 1 = *O*-desmethyldeacetyldiltiazem; 2 = deacetyldiltiazem; 3 = diltiazem; 4 = deacetyl-*N*-monodesmethyldiltiazem; 5 = *N*-monodesmethyldiltiazem; and 6 = propranolol.

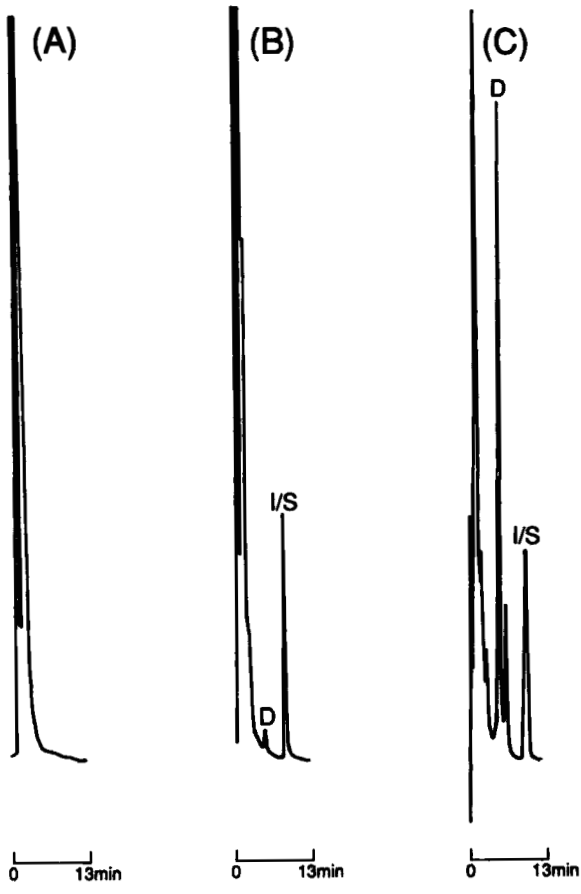

FIGURE 2

Typical chromatograms of extracts of human plasma: (A) blank plasma; (B) plasma spiked with 5 ng/ml diltiazem; and (C) plasma with 90 ng/ml diltiazem from a subject 5 hours after an oral dose of 180 mg diltiazem.

Peaks: D = diltiazem; I/S = internal standard (propranolol). In figure 2C, a peak with a retention time of 6.7 min (*i.e.* after diltiazem) was identified as deacetyl-*N*-monodesmethyldiltiazem, a metabolite of diltiazem which is commonly found in human plasma (7).

TABLE 1

Within-day Reproducibility and Accuracy of the Assay for Diltiazem in Plasma

Spike Concentration (ng/ml)	Observed Concentration[1] (ng/ml)	C.V. (%)	Accuracy[2] (%)
1	1.09 ± 0.078	7.2	109
10	10.16 ± 0.88	8.7	102
100	100.4 ± 5.7	5.7	100
500	498.4 ± 3.9	0.8	99.7

[1] Results given are mean ± S.D. (n = 5)

[2] Accuracy (%) = $\frac{\text{observed concentration}}{\text{spiked concentration}} \times 100$

the compounds of interest are: *O*-desmethyl-deacetyldiltiazem (3.7 min); deacetyl-diltiazem (5.2 min); diltiazem (6.0 min); deacetyl-*N*-monodesmethyldiltiazem (6.6 min); *N*-monodesmethyldiltiazem (7.2 min) and propranolol (10.5 min).

Figure 2 shows chromatograms of blank (drug-free) plasma, plasma spiked with 5 ng/ml of diltiazem and a typical subject's plasma chromatogram 5 hours after a single oral dose (180 mg) of diltiazem. Under these chromatographic conditions, no endogenous sources of interference were observed. More than 20 human blank plasma samples were analysed in the study and no endogenous peaks with retention time similar to diltiazem, and the internal standard, propranolol, were detected.

The mean recovery of diltiazem from plasma was 95.0 ± 10% (S.D.) at 10 ng/ml and 95.7 ± 8% at 100 ng/ml (n = 6). Recovery of the internal standard was also satisfactory at the concentration used with a recovery of 82 ± 5% (n = 6).

The standard curve of diltiazem was linear over the concentration range of 1 to 500 ng/ml with the square of the correlation coefficient (r^2) greater than 0.99. The typical linear relationship for the calibration curve can be expressed by the equation: $y = 0.0204x$; where y is the peak ratio and x is the plasma diltiazem concentration (ng/ml). The intercept (a) in all calibration curves were statistically insignificant ($p > 0.1$) and were thus not included for the calculations. The day-to-day coefficient of variation (C.V.) of the slope of the standard curves of diltiazem was 4.7% (n = 8).

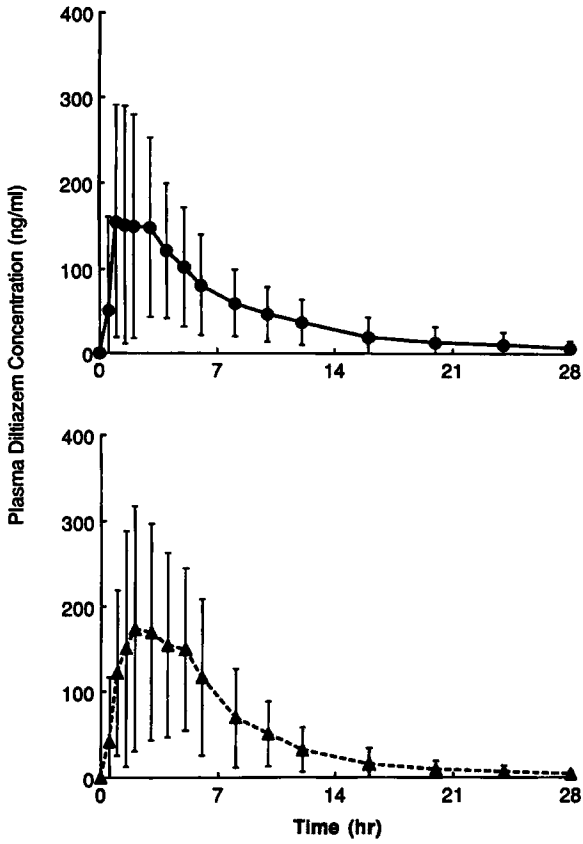

FIGURE 3

Mean plasma concentration of diltiazem *vs* time in 18 healthy male subjects after an oral administration of 180 mg diltiazem. Subjects ingested either an oral dose of a new formulation of diltiazem, *Dil-DP* (Douglas Pharmaceuticals Ltd, New Zealand, *upper panel*) or *Cardizem* (ICI, Australia, *lower panel*). The vertical bars represent the S.D. of the mean.

TABLE 2

Pharmacokinetic Parameters of Diltiazem for the New Tablet Formulation, 60 mg *Dil-DP* (Douglas Pharmaceuticals Ltd, New Zealand) and 60 mg *Cardizem* Tablets (ICI, Australia)

Parameter	3 x 60 mg Diltiazem Tablets		ANOVA
	Dil-DP	*Cardizem*	
C_{max} (ng/ml)	191 ± 126*	212 ± 135	p = 0.09
t_{max} (hr)	2.1 ± 1.1	3.1 ± 1.4	p = 0.01
$t_{1/2}$ (hr)	5.8 ± 1.3	4.8 ± 0.8	p = 0.1
AUC (ng.hr/ml)	1354 ± 1224	1427 ± 1145	p = 0.2
Fr**	0.94 ± 0.17	-	-

* Data given are mean ± S.D. (n = 18)

** $Fr = AUC_{Dil\text{-}DP}/AUC_{Cardizem}$

The within-day (within-run) reproducibility and accuracy of the diltiazem assay are presented in Table 1. At all concentrations studied the C.V. was less than 9%. These results indicate good precision of the assay. The measured value for five replications of 1 ng/ml plasma diltiazem standards gave values of 0.99, 1.18, 1.12, 1.13 and 1.03 ng/ml. This data gives a mean and S.D. of 1.09 ± 0.078 ng/ml. The C.V. of the assay at this concentration was 7.2% with accuracy of 109%. This C.V. is lower than the generally accepted C.V. of 20% for minimum quantifiable concentration (MQC). Thus, the MQC or the detection limit of sensitivity for this assay was assigned at 1 ng/ml.

Plasma samples stored at -70°C for up to 4 months showed no signs of decomposition and there was no difference in the plasma diltiazem concentrations between the fresh plasma samples and the stored plasma samples ($n = 5$, $p > 0.2$). This indicates that diltiazem is stable under these storage conditions for at least 4 months.

The present method was used to analyse the diltiazem concentrations in plasma samples collected from subjects who participated in the bioavailability study of a new formulation of diltiazem tablets, *Dil-DP*. Mean plasma diltiazem

concentration-time profiles following an oral administration of either 3 x 60 mg *Dil-DP* tablets or 3 x 60 mg *Cardizem* tablets are shown in Figure 3. The comparison between the two tablet formulations in respect to the peak plasma diltiazem concentration (C_{max}), the time to reach the maximum concentration (t_{max}), elimination half-life ($t_{1/2}$), area under the plasma concentration-time curve (AUC), and the relative bioavailability (Fr) can be seen in Table 2. Results obtained show no significant difference in AUC and C_{max} between the two formulations (ANOVA and paired *t-test*, $p > 0.05$). These results suggest that the 60 mg *Dil-DP* tablets are bioequivalent to 60 mg *Cardizem* tablets. In this study, the plasma samples were collected up to 28 hours (*i.e.* approximately 5 half-lives) after a single oral dose of 180 mg diltiazem. None of the samples had a concentration of diltiazem below the detection limit of 1 ng/ml, the lowest concentration observed at 28 hours was 1.5 ng/ml.

In summary, a sensitive HPLC method has been described for the quantitative analysis of diltiazem in human plasma. The procedures are simple, less time-consuming and provides a sufficient sensitivity for the determination of diltiazem. The assay has been shown to be suitable for use in pharmacokinetic studies.

REFERENCES

1. Echizen H. and Eichelbaum M. Clin. Pharmacokinet. **11**, 425, 1986.
2. Abernethy D.R., Schwatz J.B. and Todd E.L. J. Chromatogr. **342**, 216, 1985.
3. Clozel J.P., Caille Y., Taeyamans P., Theroux P.B. and Besner J.G. J. Pharm. Sci. **73**, 207, 1984.
4. Verghese C., Smith M.S., Aanonsen L., Pritchett E.L.C. and Shand D. J. Chromatogr. **272**, 149, 1983.
5. Wiens R.E., Runser D.J., Lacz J.P. and Dimmitt D.C. J. Pharm. Sci. **73**, 188, 1984.
6. Zoest A.R., Hung C.T., Lam F.C., Taylor R.B. and Wanwimolruk S. J. Liq. Chromatogr. (1991 - submitted).

7. Yeung P.K.F., Mosher S.J., Klassen G.A. and McGilveray I.J. Ther. Drug Monitor. **13**, 369,1991.

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