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Note

Solid-phase extraction of diltiazem and its metabolites from plasma prior to high-performance liquid chromatography

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Diltiazem is a calcium channel blocker widely used in the treatment of variant angina, hypertension and tachyarrhythmias [1]. Several high-performance liquid chromatographic (HPLC) methods have been developed for the determination of diltiazem and its metabolites in plasma samples [2–9]. However, most of these methods involve laborious liquid–liquid extractions with diethyl ether followed by acidic back-extraction, which is time-consuming.

This paper describes a rapid and selective method for the determination of diltiazem and its metabolites in plasma using solid-phase extraction prior to HPLC analysis.

EXPERIMENTAL

Reagents

Diltiazem, desacetyldiltiazem, N-demethyldiltiazem, N-demethyldeacetyldiltiazem and propionyldeacetyldiltiazem (internal standard) were generously supplied by the Clinical Research Department, Synthelabo-Recherche (L.E.R.S.) (Paris, France).

Acetonitrile, ammonium dihydrogenphosphate and orthophosphoric acid

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were obtained from Merck (Nogent-Sur-Marne, France). Triethylamine was purchased from Fluka (Buchs, Switzerland).

Apparatus and chromatographic conditions

The liquid chromatograph consisted of a Model 510 HPLC pump equipped with a Model 481 variable-wavelength absorbance detector, a Model 712 WISP sample processor (all from Waters, Saint Quentin les Yvelines, France) and a D-2000 chromato-integrator (Merck, Nogent-sur-Marne, France).

The chromatographic column was a Nucleosil C_{18} column (150 mm × 4.6 mm I.D., 3 μm particle size) preceded by a Nucleosil C_{18} guard-column (15 mm × 4.6 mm I.D.) (Interchim, Montluçon, France). The mobile phase was acetonitrile–0.1 *M* ammonium dihydrogenphosphate buffer (37:63, v/v) containing 0.08% triethylamine. The pH of the final solution was adjusted to 5.9 with orthosphosphoric acid. The flow-rate was 1.0 ml/min and the detection wavelength was 237 nm.

The extraction system consisted of solid-phase extraction columns, 100 mg

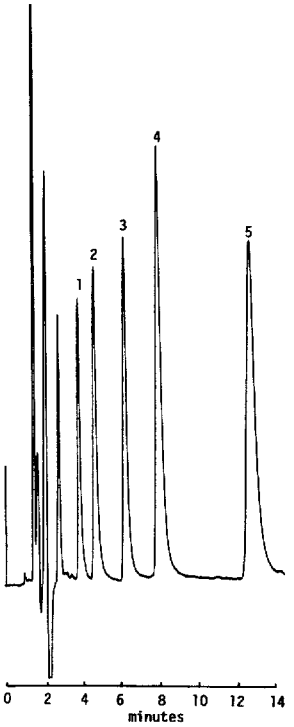

Fig. 1. Chromatogram of a standard solution containing (1) N-demethyldeacetyldiltiazem (M2), 50 ng/ml, (2) desacetyldiltiazem (M1), 50 ng/ml, (3) N-demethyldiltiazem (MA), 90 ng/ml, (4) diltiazem (DTZ), 150 ng/ml, and (5) propionyldeacetyldiltiazem, 250 ng/ml. Chromatographic conditions are described in Experimental.

of sorbent (Syva® solid-phase column, Syva-BioMérieux, Dardilly, France) and the Extra-Sep vacuum manifold for solid-phase extraction (Touzart et Matignon, Vitry-sur-Seine, France).

Sample collection and storage

Blood samples were collected in a heparinized tube and centrifuged without delay at low temperature (4°C). After centrifugation, plasma samples were immediately stored at −20°C until analysis.

Solid-phase extraction

Extraction columns were attached to the vacuum manifold. A mild vacuum was applied to the manifold to carry out the various steps of the extraction procedure.

The columns were activated by washing with two 3-ml portions of acetonitrile followed by two 3-ml portions of 0.1 *M* ammonium dihydrogenphosphate buffer. Plasma samples (1 ml) containing 1 ml of acetonitrile–0.1 *M* ammonium dihydrogenphosphate (37:63, v.v) and spiked with 50 µl of internal stan-

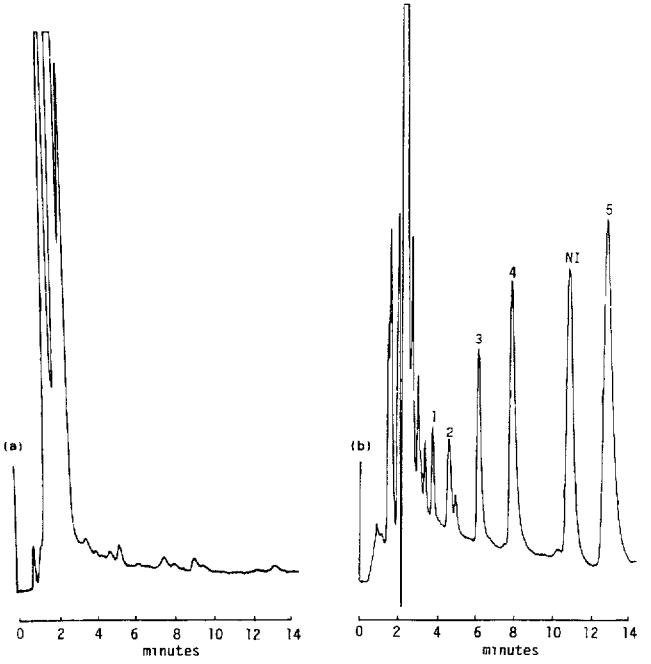

Fig. 2. Chromatograms of (a) blank plasma and (b) a plasma sample from a patient under chronic diltiazem therapy (60 mg twice daily). Injection volume, 60 µl. Peaks: 1 = N-demethyldeacetyldiltiazem (M2), 16 ng/ml; 2 = desacetyldiltiazem (M1), 15 ng/ml; 3 = N-demethyldiltiazem (MA), 50 ng/ml; 4 = diltiazem, 90 ng/ml; 5 = propionyldeacetyldiltiazem, 250 ng/ml; NI = peak not identified.

dard (propionyldeacetyldiltiazem, 5 mg/l) were then passed through the columns. The columns were washed with two 1-ml portions of acetonitrile–water (20:80, v/v) followed by two 500-μl portions of acetonitrile–water (40:60, v/v).

At this point, the columns were air-dried for 1 min. The needles were rinsed, then the compounds of interest were eluted with 500 μl of acetonitrile–0.1 *M* ammonium dihydrogenphosphate (80:20, v/v) containing 0.06% triethylamine. The eluates were evaporated to dryness under nitrogen at 40–45 °C. The residue was reconstituted with 100 μl of mobile phase and an aliquot of 60 μl was analysed by HPLC.

RESULTS AND DISCUSSION

A chromatogram of a standard solution containing diltiazem, desacetyldiltiazem, N-demethyldiltiazem, N-demethyldeacetyldiltiazem and the internal standard is shown in Fig. 1. Fig. 2 shows typical chromatograms of a blank plasma and a plasma sample from a patient under chronic diltiazem therapy.

Analytical recovery of each component was determined by addition of known concentrations of diltiazem and metabolites to plasma. The solid-phase ex-

TABLE I

PRECISION, ACCURACY AND RECOVERY

Compound[a]	Concentration added (ng/ml)	Concentration found (ng/ml)	C.V. (%)	Recovery (%)	C.V. (%)
Intra-assay (n=5)					
M2	25	24.7	7.9	87.3	6.5
	150	154.7	5.6	82.4	7.7
M1	25	23.6	3.0	83.0	3.4
	150	154.0	6.0	82.6	6.5
MA	25	23.1	4.7	85.3	4.3
	150	149.5	3.8	84.8	6.2
DTZ	25	24.4	4.2	87.3	4.6
	150	147.2	2.0	83.4	4.6
Inter-assay (n=5)					
M2	25	24.8	4.9	83.4	7.2
	150	152.0	2.5	83.6	6.6
M1	25	24.7	3.7	82.8	3.7
	150	155.2	1.7	82.8	6.4
MA	25	23.7	5.5	86.2	3.9
	150	146.8	2.3	83.0	5.5
DTZ	25	25.9	6.0	86.4	4.8
	150	152.2	4.3	82.6	4.4

[a]For abbreviations see legend to Fig. 1.

traction and analyses were then performed as described and the peak heights compared with those obtained by direct injection of aqueous standards. Mean recoveries at 25 and 150 ng/ml are given in Table I.

The linearity (peak height versus concentration) tested for each compound was excellent up to 300 ng/ml. The detection limits at a signal-to-noise ratio of 4 were 0.15 ng for N-demethyldeacetyldiltiazem and desacetyldiltiazem and 0.3 ng for N-demethyldiltiazem and diltiazem, with a coefficient of variation (C.V.) of 10% or less.

Reproducibility and accuracy were determined with spiked plasma samples. The intra-assay and inter-assay results are given in Table I.

During the analytical procedure, no degradation of diltiazem or its metabolites was observed.

The Nucleosil C_{18} column has demonstrated a long lifetime; ca. 700 samples were injected without any deterioration of its performance.

There was no interference with the compounds of interest by other drugs that are often co-administered with diltiazem: diazepam, midazolam, flunitrazepam, pancuronium bromide, quinidine, verapamil, nifedipine, propranolol, procainamide.

Solid-phase extraction represents a rapid and reliable sample preparation method for the determination of diltiazem and its metabolites in plasma samples compared with the conventional liquid–liquid extraction, which involves laborious extraction steps with diethyl ether at alkaline pH followed by acidic back-extraction [2–4,6–8]. Moreover, the extraction efficiency of the solid-phase extraction procedure is superior to most of the conventional liquid–liquid extraction methods developed for the analysis of diltiazem and its major metabolites in plasma [3,4,7].

In conclusion, solid-phase extraction prior to HPLC represents a convenient method for quickly obtaining clean extracts with optimum recoveries, and will be an accurate analytical tool for the monitoring of diltiazem and metabolite concentrations in plasma samples during pharmacokinetic studies.

REFERENCES

1 M. Chaffman and R.N. Brodgen, Drugs, 29 (1985) 387.
2 C. Verghese, M.S. Smith, L. Aanonsen, E.L.C. Pritchett and D.G. Shand, J. Chromatogr., 272 (1983) 149.
3 K.J. Goebel and E.U. Kolle, J. Chromatogr., 345 (1985) 355.
4 P. Höglund and L.G. Nilsson, J. Chromatogr., 414 (1987) 109.
5 S.C. Johnson and S.K.W.K. Khalil, J. Liq. Chromatogr., 10 (1987) 673.
6 S.C. Montanat, D.R. Abernethy and J.R. Mitchell, J. Chromatogr., 415 (1987) 203.
7 L.M. Dubé, N. Mousseau and L.J. McGilveray, J. Chromatogr., 430 (1988) 103.
8 H. Zhao and S. Chow, Pharm. Res., 6 (1989) 458.
9 V. Ascalone and F. Flaminio, J. Chromatogr., 495 (1989) 358.