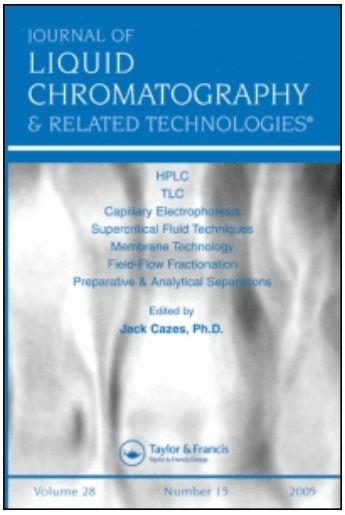

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Comparison of Solid-Phase Extraction and Liquid-Liquid Extraction Methods for Liquid Chromatographic Determination of Diltiazem and its Metabolites in Plasma

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Original Article

COMPARISON OF SOLID-PHASE EXTRACTION AND LIQUID-LIQUID EXTRACTION METHODS FOR LIQUID CHROMATOGRAPHIC DETERMINATION OF DILTIAZEM AND ITS METABOLITES IN PLASMA

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ABSTRACT

A rapid solid-phase extraction method for the determination of diltiazem and its metabolites from plasma was compared to a conventional liquid-liquid extraction procedure we have described previously. Analytical recovery for all compounds was greater than 90 % for solid-phase extraction whereas for liquid-liquid extraction, mean recovery ranged from 67 to 82 %. The increase of extraction efficiency was closely related to an improvement of the detection limit for the metabolites. Solid phase extraction procedure was found to be more convenient, rapid and sensitive than liquid-liquid extraction and represents a useful analytical tool for the monitoring of diltiazem and its metabolites in clinical investigations.

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INTRODUCTION

Diltiazem is a calcium channel antagonist widely used in the treatment of variant angina (1). The drug is also effective as an antiarrythmic and antihypertensive agent (2).

Several high performance liquid chromatographic (HPLC) methods have been published for the determination of diltiazem and deacetyldiltiazem (M1) generally considered to be the principal metabolite in plasma samples (3-12). However, only few methods (4, 6, 9, 11, 12) permit the determination of other metabolites, particurlaly N-demethyldiltiazem (MA) which was recently found to be the major plasma metabolite in human (13, 14). Most of these methods (4-10, 12) involve laborious liquid-liquid extraction (LLE) followed by an acidic back extraction which is time consuming.

To facilitate the analysis of hundred of plasma samples generated by pharmacokinetic studies of diltiazem and its metabolites, we have developed a rapid solid-phase extraction (SPE) method of these compounds from plasma samples prior to high performance liquid chromatographic analysis (15).

In the present paper, we propose to compare this SPE procedure, slightly modified to improve the extraction efficiency and the sensitivity, to a LLE procedure we have previously described (12).

MATERIALS AND METHODS

Chemicals

Diltiazem (DTZ), deacetyldiltiazem (M1), N-demethyldiltiazem (MA), N-demethyldeacetyldiltiazem (M2) and propionyldeacetyldiltiazem used as internal standard were generously supplied by the Clinical Research Department, L.E.R.S., Paris, France.

Acetonitrile, ammonium dihydrogen phosphate, boric acid, sodium chlorid and orthophosphoric acid were obtained from Merck (Nogent-Sur-Marne, France). Triethylamine was purchased from Fluka (Buchs, Switzerland).

Apparatus and chromatographic conditions

The apparatus 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).

Chromatographic separation was achieved using a Nucleosil C 18 column (150 x 4.6 mm ID), 3 µm particle size preceded by a Nucleosil C 18 guard-column (15 x 4.6 mm ID) (Interchim, Montluçon, France). The mobile phase consisted of acetonitrile/ ammonium dihydrogen phosphate buffer (0.1 mol/L), 35/65 by vol., containing 0.08 % of triethylamine. The pH of the final solution was adjusted to 5.9 with orthophosphoric acid. The flow rate was 1 ml/min and the detection was performed at 237 nm.

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 - 80°C until analysis.

Liquid-liquid extraction procedure

Diltiazem and its metabolites were extracted from plasma using the LLE procedure we have described previously (12). Plasma (1 ml) containing 500 µl of 0.1 mol/L borate buffer and 50 µl of internal standard (propionyl deacetyldiltiazem, 5 µg/ml) was extracted with 5 ml of methyltertbutyl ether by mixing for 20 minutes. After centrifugation, the organic phase was then back-extracted with 500 µl of 2.1 mmol/L orthophosphoric acid. The acid phase was evaporated to dryness and the residue was reconstituted with 100 µl of mobile phase. An aliquot of 60 µl was injected on to the HPLC column.

Solid-phase extraction procedure

The extraction system consisted of solid-phase extraction columns, 100 mg sorbent (SYVA-BioMérieux, Dardilly, France) and the Extra-Sep vacuum manifold for solid phase extraction (Touzart et Matignon, Vitry-sur-Seine, France).

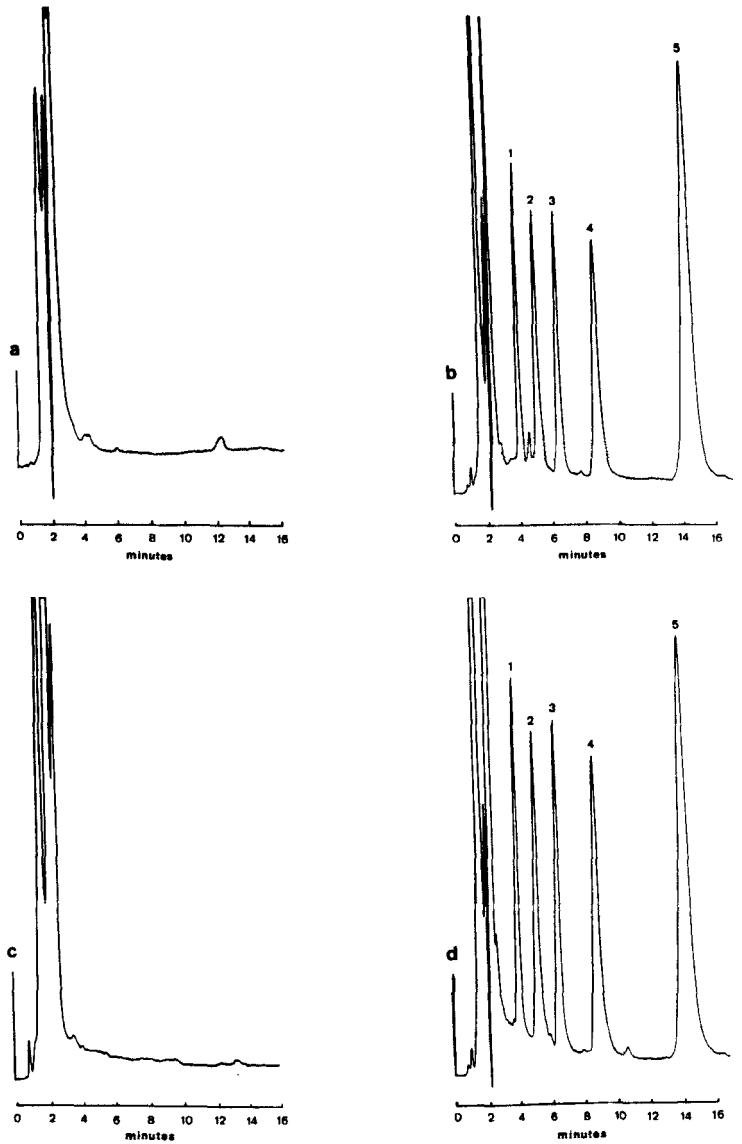

Figure 1 - Chromatograms of blank plasma from (a) LLE method, (c) SPE method and plasma spiked with diltiazem and its metabolites from (b) LLE method and (d) SPE method. Chromatographic conditions are described in materials and methods. Peaks : 1-N-demethyldeacetyldiltiazem (M2), 5O ng/ml. 2-deacetyldiltiazem (M1), 50 ng/ml. 3-N-demethyldiltiazen (MA), 75 ng/ml. 4-diltiazem, 100 ng/ml. 5-propionyldeacetyldiltiazem (internal standard), 250 ng/ml.

TABLE 1

Analytical recovery of diltiazem, its metabolites and internal standard from plasma samples extracted by SPE or LLE methods

	Concentrations ng/ml	Recovery % LLE	Recovery % SPE
M2	25	69 ± 8	97± 5
	150	67 ± 8	90 ± 6
M1	25	73 ± 6	92 ± 3
	150	74 ± 6	90 ± 6
MA	25	79 ± 6	95 ± 3
	150	74 ± 6	93 ± 5
DTZ	25	82 ± 8	97 ± 4
	150	79 ± 7	95 ± 5
IS	250	91 ± 4	99 ± 3

each value represents the mean ± SD of five determinations

The solid phase extraction columns were activated with 3 ml of acetonitrile followed by 3 ml of 0.1 mol/L ammonium dihydrogen phosphate buffer. Plasma samples (1 ml) spiked with the internal standard were diluted with 1 ml of a mixture of acetonitrile/0.1 mol/L ammonium dihydrogen phosphate (35/65 by vol.) and passed through the columns. The columns were washed with 2 ml of acetonitrile/water (20/80 by vol.) followed by 2 ml of acetonitrile/water (40/60 by vol.). Elution was performed with 500 µl of acetonitrile/0.1 mol/L ammonium dihydrogen phosphate (80/20 by vol.) containing 0.06 % of triethylamine. The eluates were evaporated to dryness

TABLE 2

Precision and accuracy of the two extraction methods

Within day (n = 5)

		LLE		SPE	
	Amount added ng/ml	Amount found ng/ml	CV %	Amount found ng/ml	CV %
M2	25	20.9	4.9	24.7	7.9
	150	142.0	2.9	154.7	5.6
M1	25	25.0	7.3	23.6	3.0
	150	142.6	1.6	154.0	6.0
MA	25	25.6	3.0	23.1	4.7
	150	147.1	1.2	149.5	3.8
DTZ	25	25.4	3.4	24.4	4.2
	150	148.3	3.6	147.2	2.0

Day-to-day (n = 5)

		LLE		SPE	
	Amount added ng/ml	Amount found ng/ml	CV %	Amount found ng/ml	CV %
M2	25	22.2	4.9	24.8	4.9
	150	151.0	4.7	152.0	2.5
M1	25	25.6	2.8	24.7	3.7
	150	148.9	3.3	155.2	1.7
MA	25	24.3	4.2	23.7	5.5
	150	148.5	2.7	146.8	2.3
DTZ	25	24.4	5.4	25.9	6.0
	150	149.8	2.0	152.2	4.3

under nitrogen at 40-45°C. The residues were reconstituted with 100 µl of mobile phase and 60 µl injected on to the column.

RESULTS AND DISCUSSION

The typical chromatograms of blank plasma and plasma standard spiked with diltiazem, its metabolites and the internal standard from LLE method and from SPE method are shown in figure 1.

Analytical recovery of each compound was determined by comparing the peak heights obtained by direct injection of aqueous standards to those obtained after plasma LLE or SPE methods. The results are given in table 1. For SPE, the average recovery for all the compounds at different concentrations was greater than 90 %, (ranging from 90 to 99 %) whereas for LLE, mean recovery was lower than 80 % for the metabolites with a recovery inferior to 70 % for M2.

Calibration curves of each compound were linear over the concentration range 5 - 300 ng/ml for both extraction methods. The minimum quantity detectable (defined as a signal-to-noise ratio of 4) was found to be 0.30 ng for each compound for LLE ; 0.15 ng for M2 and M1 and 0.30 ng for MA and DTZ for SPE with a coefficient of variation of 10 % or less. The within-day and day-to-day precision and accuracy of the two extraction methods determined by replicate analysis of plasma samples spiked with diltiazem and its metabolites are given in table 2. The within-day and day-to-day precision of the two methods were comparable.

Specificity of the methods was evaluated in the presence of others drugs which are often coadministered with diltiazem : diazepam, flunitrazepam, midazolam, nifedipine, pancuronium bromide, procaïnamide, propranolol, quinidine, verapamil. None of these substances interfered with or influenced the determination of diltiazem and its metabolites.

In contrast to LLE procedure, SPE method leads to an improvement of analytical recovery which is closely related to an improvement of detection limit

especially for M2 and M1 metabolites which are found at low levels in plasma from patients under diltiazem therapy.

Furthermore, using SPE procedure, there is no risk of degradation of diltiazem and its metabolites due to stressing conditions such as acidic or basic conditions as reported by some authors (15).

In conclusion, solid-phase extraction represents a more convenient, rapid and sensitive method than liquid-liquid extraction procedure to obtain clean plasma extracts with optimum recoveries for the monitoring of diltiazem and metabolites in clinical investigations.

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