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Solid-phase extraction of naftidrofuryl from human plasma for high-performance liquid chromatography analysis

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Summary

A solid-phase extraction method is described for isolating naftidrofuryl from human plasma spiked with verapamil hydrochloride as internal standard. Chromatographic analysis of the eluate was accomplished using a reversed phase (SAS Hypersil) column and a mobile phase (pH 7.3) consisting of acetonitrile, 0.5% (w/v) KH_2PO_4 and diethylamine (50:50:0.05, by vol.). The limit of detection by using a fluorimetric detector was 0.6 ng ml^{-1}. Recoveries were in the interval 96.6–101.4% over the plasma concentration range from 4 to 300 ng ml^{-1}. Precision analysis indicated an RSD between 1.1 and 3.0% for the method at plasma concentrations ranging from 10 to 300 ng ml^{-1}.

Introduction

Naftidrofuryl hydrogen oxalate (Fig. 1) is claimed to be a smooth muscle relaxant and has the ability to increase peripheral and cerebral blood flow (Fontaine et al., 1968, 1969). It has been shown that it enhances tissue oxidative metabolism (Meynaud et al., 1973; Shaw and Johnson, 1975).

Several methods have been described for determination of the substance in plasma. Most methods utilize high-performance liquid chromatography (HPLC) determination with detection either via ultraviolet analysis (Brodie et al., 1979; Garrett and Barbhaiya, 1981; Beyer and Hildebrand, 1982) or by fluorimetry (Beyer and Hildebrand, 1982; McKnight, 1989). Gas chromatography (Beyer and Hildebrand, 1982) and direct fluorimetric detection using a fluorescence indicator (Belleville and Lechevin, 1968) have also been used. The isolation methods employed are liquid/liquid (Brodie et al., 1979; Garrett and Barbhaiya, 1981; Beyer and Hildebrand, 1982; McKnight, 1989), as well as solid-phase extrac-

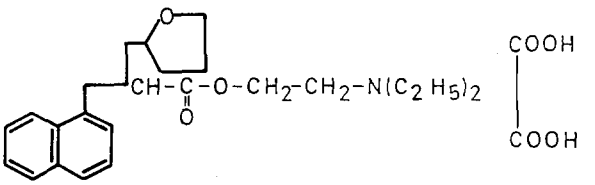

Fig. 1. Chemical structure of naftidrofuryl hydrogen oxalate.

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tions (SPE) (Beyer and Hildebrand, 1982). However, recoveries of less than 83% were reported for the application of Extrelut™ columns in the latter method.

SPE columns are being used to an ever-increasing extent due to both the analytical selectivity and the efficacy of sample pre-treatment. Compared to liquid/liquid extraction, SPE is simple and reduces the requirements for large volumes of organic solvents.

The present study describes a method for isolating naftidrofuryl from plasma by means of SPE, followed by quantification using HPLC.

Materials and Methods

Materials

Naftidrofuryl hydrogen oxalate (Co-Med, Hamburg, Germany) was used as received. Verapamil hydrochloride (Knoll, Ludwigshafen, Germany) was used as internal standard (McKnight, 1989). Acetonitrile, methanol and diethylamine (Merck, Darmstadt, Germany) were of HPLC grade, and sodium acetate and KH_2PO_4 (Merck) were of analytical grade. Double-distilled water was used throughout the study.

Sample preparation

Samples of drug-free human plasma were spiked with naftidrofuryl hydrogen oxalate to yield concentrations between 4 and 300 ng ml^{-1}. To 1.00 ml aliquots of the spiked plasma, 100 μl aqueous solution of the internal standard (2 μg ml^{-1}) was added.

Standard aqueous solutions were prepared by dissolving naftidrofuryl hydrogen oxalate in water, yielding concentrations of 10, 50 and 300 ng ml^{-1}, and spiked with the same amount of internal standard.

Extraction

1 ml Bakerbond™ C_2 (Baker, Phillipsburg, NJ, U.S.A.) SPE columns were inserted into an extraction system (Model 10, Baker) through aluminium valves, equipped with stainless-steel syringes. The columns were conditioned with 2 ml of methanol followed by 2 ml of water, under gentle vacuum. Without allowing the columns to dry, 1.00 ml plasma samples spiked with internal standard were added to the extraction columns, and aspirated through the columns. The columns were then washed with four 1 ml aliquots of water. The samples were eluted twice with 250 μl aliquots of a mixture of methanol and 0.06 mol l^{-1} acetate buffer, pH 3.0 (70:30, v/v), and the eluates were collected in glass vials.

HPLC procedures

The HPLC system consisted of a pump (Model 300B, Gynkotek, München, Germany) connected to a Hypersil SAS column (250 × 4.6 mm) (Techlab, Erkerode, Germany), a guard column (40 × 4.6 mm) (Techlab), and an autosampler (Model 465, Kontron, München, Germany), equipped with a 200 μl loop. Detection was performed by means of a fluorescence spectromonitor detector (Model RF-530, Gynkotek) operating at wavelengths of $\lambda = 276$ nm (excitation) and $\lambda = 334$ nm (emission). Data were processed with the aid of a 386 PC system equipped with a coprocessor, using the DS 450-MT software (Kontron). The mobile phase (pH 7.3) consisted of acetonitrile, 0.5% (w/v) KH_2PO_4 and diethylamine (50:50:0.05, by vol.), and was degassed before use. The flow rate was 2.0 ml min^{-1} giving a pressure of 120 bar.

The selectivity analyses of naftidrofuryl and verapamil were performed on spiked plasma samples by means of diode array detection. The HPLC system consisted of a two-piston pump (Waters 600, Milford, MA, U.S.A.), a controller (Waters 600 E), and a photodiode array detector (Waters 991). The stationary and mobile phases as well as the operating conditions were the same as those of the normal HPLC procedure.

Degradation products

A standard aqueous solution of naftidrofuryl hydrogen oxalate (100 μg ml^{-1}) and verapamil hydrochloride (100 μg ml^{-1}) was boiled under reflux in a 0.1 N sodium hydroxide solution for 2 h. The solution was diluted before injection into the HPLC system.

Results and Discussion

Selectivity

The blank biological matrix demonstrates no interfering peaks with the peaks of either verapamil or naftidrofuryl (Fig. 2). Baseline separation is observed between the two main peaks, which have retention times of 6.7 and 8.3 min, respectively, and the degradation products. The selectivity was further evaluated by diode array analysis, which demonstrated the absence of matrix interference.

Linearity

Plasma-free calibration curves for naftidrofuryl showed good linearity ($R^2 > 0.9995$) between peak area and amount. The curves were constructed from duplicate measurements of seven standards in the range 12.5–125 ng ml^{-1}. The intercepts were not significantly different ($\alpha = 0.05$) from

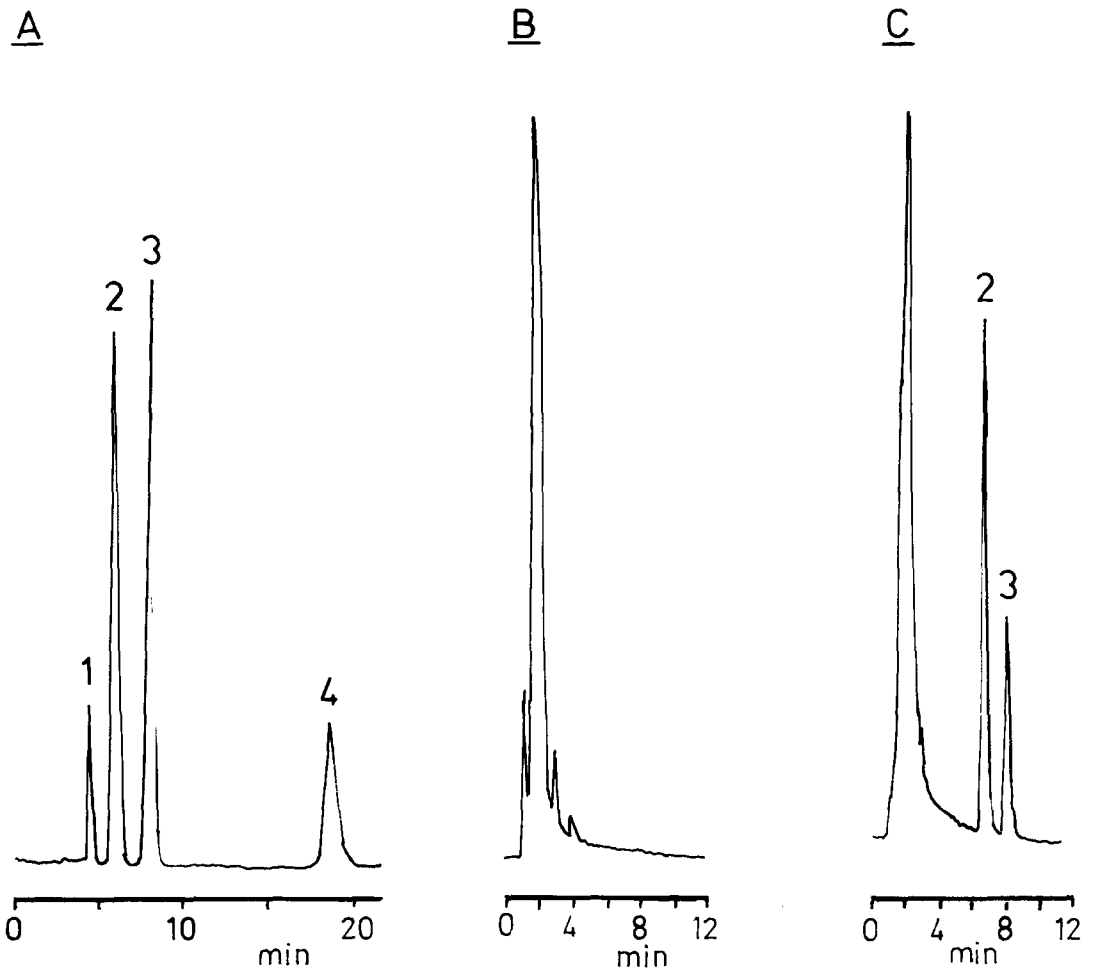

Fig. 2. Chromatograms of (A) standard solution and its degradation products, (B) a blank plasma sample and (C) plasma spiked with 50 ng ml^{-1} naftidrofuryl hydrogen oxalate and 200 ng ml^{-1} verapamil hydrochloride. Peaks: 1, degradation product of verapamil hydrochloride; 2, internal standard (verapamil); 3, naftidrofuryl; 4, degradation product of naftidrofuryl hydrogen oxalate.

zero. Residual vs concentration plots indicated no heterocedasticity.

Precision

The estimation of variabilities due to differences in both the SPE columns and HPLC system was performed at three concentrations. 10 samples from the same spiked sample were extracted, injecting each sample in triplicate and measuring the peak areas.

The variance of the whole method is the sum of those due to the inter- and intra-assay variability. These values can readily be estimated, since the total sum of squares of all injections is the sum of the sum of squares due to sample preparation and to the HPLC system. At high concentrations, the magnitude of the estimated relative standard deviation (RSD) for the method is comparable with that for the system (Table 1). At lower concentrations, the RSD of both the system and the method increases. In addition, the differences between the values become larger as a consequence of greater variability in the extraction procedure and in the HPLC system.

The precision of the system without the influence of plasma was determined by injecting a standard aqueous solution 10 times.

At the lowest concentration, the RSD of the system without plasma is equal to that of the method. At the other concentrations examined, the values approximate the RSD for the HPLC system with plasma. If the introduction of the plasma component has little effect on the precision of the HPLC system, the RSD values with and without plasma should be identical. The discrepancy observed at the lowest concentration measured can be explained since relatively small absolute deviations about the mean at this concentration increase the absolute variance dramatically.

TABLE 1

Data on the precision of the system and method analysis ($n = 3$, $a = 10$)

Concentration (ng ml^{-1})	Without plasma (% RSD)	With plasma	
		System (% RSD)	Method (% RSD)
10	3.3	1.5	3.0
50	1.2	1.3	2.5
300	0.6	0.8	1.1

TABLE 2

Accuracy of the system ($n = 10$)

Concentration (ng ml^{-1})	Absolute recovery (%)	Confidence interval on the recovery ($\alpha = 0.05$)
4	99.8	$96.7 \leq \mu \leq 102.9$
8	100.9	$97.8 \leq \mu \leq 104.0$
40	101.4	$100.6 \leq \mu \leq 102.2$
100	96.7	$95.5 \leq \mu \leq 97.9$
300	96.6	$96.1 \leq \mu \leq 97.1$

Accuracy

The accuracy of the method was determined by mixing the eluate from three extractions of the same spiked plasma sample and injecting the sample 10 times. According to Table 2, the absolute recovery of the drug lies within $\pm 3\%$ of the true values, which should be considered as satisfactory.

Binding of naftidrofuryl (Fontaine et al., 1969), as well as verapamil (Hamann et al., 1984; Somani et al., 1987), to plasma proteins has been reported. Quantitative errors due to losses of substances which had bound to proteins could therefore be expected. The influence of the plasma component on the recoveries was evaluated by comparing the recoveries measured on spiked plasma samples and aqueous solutions. Standard aqueous solutions were prepared according to the method described above. A two-sided t-test ($\alpha = 0.05$), in order to confirm no difference between the treatments, showed no significant matrix effect on the recovery.

Analytical stability

The solutions were assayed by mixing the eluate from four extractions of the same spiked sample, injection being performed in triplicate. Two parallel samples of each concentration (50 and 300 ng ml^{-1}) were prepared. The solutions were stored at 4°C and at room temperature, and were withdrawn after 2 and 4 days.

Aqueous solutions of naftidrofuryl have been shown to be most stable at pH 3.0 (Garrett and Barbhaiya, 1981). An instability in the acidic elution medium is therefore not expected. This was demonstrated by an analysis of variance which showed no significant decrease in the recovery after storage ($\alpha = 0.05$). Furthermore, no significant dependence on the storage conditions and concentrations was found.

Sensitivity

The limit of detection (LOD), calculated according to a signal/noise ratio of 3 (Long and Winefordner, 1983), was 0.6 ng ml^{-1}, which was the lowest concentration which could be distinguished from the blank plasma. The limit associated with reliable quantification (LOQ) was estimated to 2 ng ml^{-1}.

Conclusions

The sample preparation and analytical method described in the present paper is suitable for accurate isolation and quantification of naftidrofuryl from human plasma in the concentration range from 4 to 300 ng ml^{-1}. The method shows acceptable precision limits and limit of quantification, and is applicable to routine drug monitoring.

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