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Note

High-performance liquid chromatography of naftopidil, a novel antihypertensive drug, and two metabolites in human plasma

GEORG NIEBCH* and HARALD O. BORBE

Department of Biochemistry, ASTA Pharma AG, P O Box 100105, D-6000 Frankfurt (F.R G)

and

EBERHARD BESENFELDER

Department of Biochemistry, Boehringer Mannheim GmbH, P O Box 310120, D-6800 Mannheim (F R G)

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Naftopidil, *RS*-1-[4-(2-methoxyphenyl)-1-piperazinyl]-3-(1-naphthoxy)-2-propanol, is a novel antihypertensive drug currently under clinical investigation [1]. Studies of its mode of action indicate that it is a selective α_1-adrenoceptor antagonist [2,3] with Ca^{2+}-antagonistic properties [3] and a pronounced affinity for 5-HT_{1A} receptors [2].

Studies of the metabolism of naftopidil in animals and humans revealed two major metabolites, O-desmethylnaftopidil and (phenyl)hydroxynaftopidil (unpublished observations), each with an affinity for α_1-adrenoceptors similar to that of the parent compound [2].

This paper describes a rapid high-performance liquid chromatographic (HPLC) assay with fluorescence detection for the quantitative determination of naftopidil and its two active metabolites in blood plasma, using an internal standard.

EXPERIMENTAL

Reagents and chemicals

Naftopidil (base) and the internal standard carvedilol (base) were supplied by Boehringer Mannheim (Mannheim, F.R.G.). O-Desmethylnaftopidil·2HCl and (phenyl)hydroxynaftopidil·2HCl were synthesized at ASTA Pharma (Frankfurt, F.R.G.). Their structures are shown in Fig 1. All other chemicals and solvents were purchased in analytical-grade quality from E. Merck (Darmstadt, F. R. G.).

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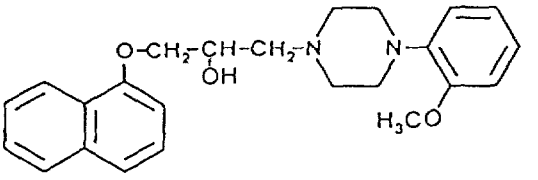
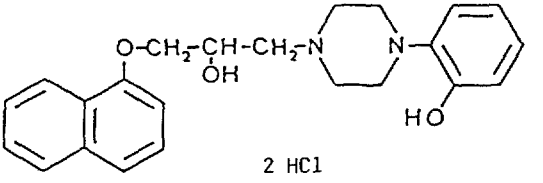
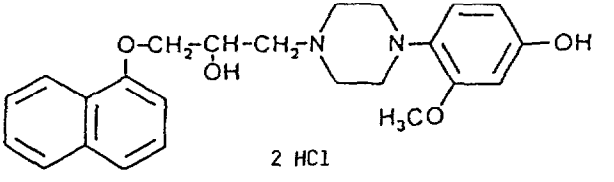
Naftopidil (base)



O-desmethyl-naftopidil·2 HCl



(phenyl)Hydroxy-naftopidil·2 HCl



Carvedilol (base, internal standard)

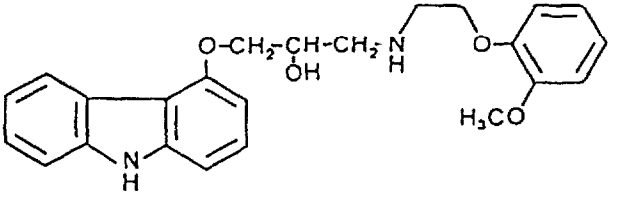


Fig 1 Structures of naftopidil, its analysed metabolites and the internal standard.

Instrumentation and chromatographic conditions

The HPLC equipment consisted of two Model 510 pumps, a WISP 712 injector, a SIM interface and an IBM XT as workstation with Maxima 820 software (all from Waters, Eschborn, F.R.G.). Columns (250 mm × 4.6 mm I.D.) packed with 5-μm RP-LiChrosorb Select B (Hibar) were supplied by E. Merck. The mobile phase components were 0.02 *M* KH_2PO_4 (adjusted to pH 1.8 with phosphoric acid) (A) and acetonitrile-methanol (1:1, v/v) (B). They were mixed in the ratio A:B = 55:45. The flow-rate was 0.8 ml/min.

A Spectroflow 980 fluorescence detector (ABI-Kratos, Weiterstadt, F.R.G.) was used with the following settings: excitation wavelength, 215 nm; emission wavelength, above 320 nm.

Plasma extraction procedure

A 1-ml plasma sample was fortified with 50 ng of the internal standard carvedilol (10 μl methanolic solution), alkalinized by addition of 500 μl of phosphate buffer (1 *M* K_2HPO_4, pH 9.2) and extracted with 8 ml of diethyl ether for 10 min on a rotary mixer (Reax from Heidolph, Kelheim, F.R.G.). Then 5 ml of the organic phase were back-extracted into 300 μl of 0.05 *M* sulphuric acid for 10 min on the rotary mixer. Following a short centrifugation at 1000 *g*, the diethyl ether phase was removed by aspiration. After a 30-s treatment with a stream of nitrogen, 250 μl of the acidic aqueous extract were removed and mixed with 50 μl of phosphate buffer (as above). Aliquots of 100 μl were injected and analysed by HPLC.

Calibration

Calibration lines were prepared in the range 1–150 ng/ml by processing 1 ml of control plasma spiked with 10 μl of the respective methanolic analyte solutions and with 10 μl (50 ng) of the methanolic internal standard solution.

RESULTS AND DISCUSSION

Naftopidil and its active O-desmethyl and (phenyl)hydroxy metabolites all exhibit native fluorescence. Owing to their basic character they can be isolated from the plasma matrix by extraction with diethyl ether at alkaline pH and acidic back-extraction. From the chromatograms the peak-area ratios relative to the internal standard can be evaluated.

The separation is based on reversed-phase chromatography. An enantiomeric separation (all analytes are racemates) was not developed as *R*- and *S*-naftopidil were shown to be almost equal pharmacologically active [3].

Linearity and recovery

The assay is linear for naftopidil and its active metabolites in the concentration range 1–150 ng/ml. Typical calibration-line equations are as follows:

For naftopidil: concentration = −0.332 967 + 73.765 74 (area ratio); r^2 = 0.9997.

For O-desmethylnaftopidil·2HCl: concentration = −0.090 875 + 80.485 49 (area ratio); r^2 = 0.9998.

For (phenyl)hydroxynaftopidil·2HCl: concentration = 0.046 030 + 111.4514 (area ratio); r^2 = 0.9997.

The deviations at individual calibration points between spiked and calculated concentrations are always less than ±10%. The numerical recoveries of the spiked analytes, after correction for the aliquot losses, amount to 83% (naftopidil), 93% (hydroxynaftopidil), 89% (desmethylnaftopidil) and 97% (internal standard).

No attempt was made to improve the extractability of the analytes from plasma by buffering at different pH values.

Precision

The coefficients of variation for serial determinations of the three compounds at 2, 25 and 100 ng/ml were found to be equal to or less than 6% (Table I).

Limit of quantitation

Despite the accuracy reported above, the calibration values of 1 ng/ml often exhibited deviations exceeding ± 10%. Thus, 2 ng/ml was regarded as the reliable lower limit of quantitation for all three analytes.

Storage stability

Plasma samples from a clinical study were frozen again after a first analysis and reanalysed at irregular time intervals. The results indicate that the plasma samples can be stored at −18°C until analysis for up to one year

Pharmacokinetic studies in humans

Fig. 2 shows a characteristic chromatographic profile for a plasma sample taken from a healthy, fasted subject 1 h after oral administration of a 50-mg naftopidil tablet.

TABLE I

ASSAY PRECISION IN SERIAL DETERMINATIONS

Concentration (ng/ml)	n	Coeffficient of variation (%)		
		Naftopidil	Desmethylnaftopidil 2HCl	Hydroxynaftopidil 2HCl
2	5	3 9	5 0	6.0
25	5	2.9	3 7	3.3
100	6	1.3	3 1	5.1

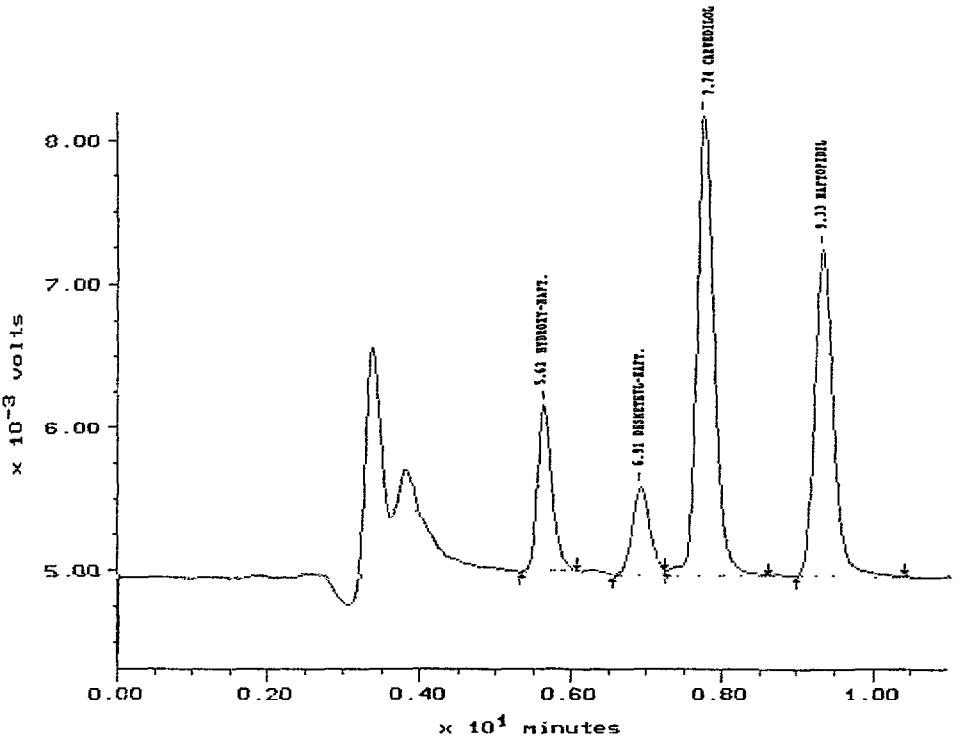


Fig 2. HPLC profile for an *in vivo* human plasma sample, taken 1 h after administration of a 50-mg tablet, during the steady state. Concentrations naftopidil, 61 4 ng/ml (156 4 n*M*), O-desmethylnaftopidil·2HCl, 16 1 ng/ml (35.7 n*M*); (phenyl)hydroxynaftopidil·2HCl, 35 8 ng/ml (74.4 n*M*)

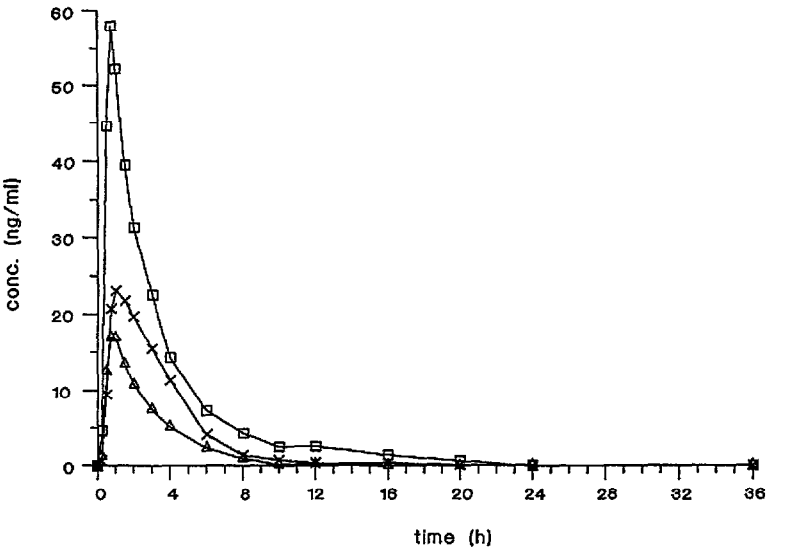


Fig 3. Mean plasma levels of naftopidil and two of its metabolites observed in twelve healthy volunteers following a single 50-mg tablet of naftopidil orally. (□) naftopidil, (△) O-desmethylnaftopidil 2HCl, (×) (phenyl)hydroxynaftopidil·2HCl.

Fig. 3. demonstrates the mean plasma level–time courses of one of the pharmacokinetic studies evaluated with this assay.

ACKNOWLEDGEMENTS

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