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Short Communication

A rapid and sensitive method for the quantitation of levoprotiline in human blood and plasma*

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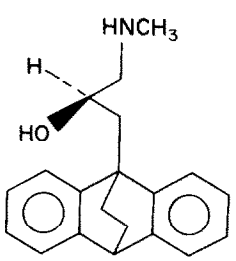
Introduction

The new tetracyclic antidepressant levoprotiline, α[(methylamino)methyl]-9,10-ethanoantracene-9(10*H*)-ethanol hydrochloride, is the *R*(−)-isomer of the racemate oxaprotiline. In contrast to the racemate and to the *S*(+)-isomer, levoprotiline is completely devoid of norepinephrine uptake inhibiting properties [1, 2]. However, in clinical studies levoprotiline displayed antidepressant effects comparable to those of the racemic compound [3]. After oral administration to man the drug is almost completely absorbed and extensively metabolized. Direct *O*-glucuronidation is the major metabolic pathway [4]. Several sensitive methods for the analysis of unchanged levoprotiline in blood or in plasma have been described recently [5–7]. Based upon the detection of derivatives, the reported procedures are rather time-consuming and therefore not altogether suitable for routine analysis. The specified sensitivity of the only direct HPLC method, using three-fold liquid–liquid extraction of plasma samples followed by UV detection [8] could not be reproduced in the authors' laboratory.



Levoprotiline

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In order to investigate the pharmacokinetics of levoprotiline in blood and plasma after low intravenous (i.v.) doses administered to young healthy volunteers, a rapid and sensitive method has been developed. The assay is performed by simple liquid–liquid extraction of the drug from the biological matrix and direct measurement by HPLC with fluorescence detection. Maprotiline, having the non-hydroxylated parent structure of levoprotiline, is used as internal standard.

Experimental

Solutions

Stock solutions of levoprotiline hydrochloride and the internal standard were prepared in methanol at a concentration of 10 ng μl^{-1}. To spike blood and plasma samples, the stock solutions were diluted with 0.01 M HCl to the concentration of 1 ng μl^{-1} (calibration solutions). Stock solutions were prepared once a month whilst the calibration solutions were made up once a week. The solutions were perfectly stable for these periods at −4°C.

Procedure for blood and plasma samples

Extraction. A 50 μl solution of the internal standard (1 ng μl^{-1}) was added to 0.5–1.0 ml plasma or homogenized blood (20 min in ultrasonic bath). After addition of 0.2 ml of 1.0 M KOH solution and 5 ml *n*-hexane/ethyl acetate (1:1, v/v) the mixture was extracted by shaking (20 min, 250 rpm). After centrifugation (10 min, 3500 rpm) the organic phase was separated and evaporated to dryness (N_2, 30°C). The residue was redissolved in 100–150 μl of mobile phase and after short stirring on a vortex mixer injected into the chromatograph (30–40 μl).

High-performance liquid chromatography

The modular system used consisted of automatic injector, reciprocating pump, and spectrofluorimetric detector (Applied Biosystems), integrator (SP 4270 Spectra-Physics). A 4 μm NOVA-PAK C18, 15 × 0.39 i.d. cm column (Waters) was used along with a Vydac 30–40 μm SC-201 RP, 3 × 0.39 cm (Machery-Nagel) (6:4, v/v) precolumn. The mobile phase consisted of 0.05 M KH_2PO_4–acetonitrile plus 0.003 M 1-octane-sulphonic acid sodium salt, pH 3.0, was supplied at 0.7 ml min^{-1}. Detection was performed using excitation and emission wavelengths of 208 and 280 nm, respectively. Under the above conditions levoprotiline and the internal standard had retention times of 5.2 and 9.2 min, respectively.

Results and Discussion

Chromatograms of human blood and plasma spiked with levoprotiline and the internal standard, as well as, the chromatograms of blood and plasma samples after i.v. administration of 10 mg levoprotiline to healthy volunteer are presented in Fig. 1. The blank control samples showed no interfering peaks.

Linearity of the method

Calibration curves were found to be linear in the range of 3–50 ng ml^{-1} levoprotiline in blood and plasma. The correlation coefficients were: $r_{plasma} > 0.999$; $r_{blood} > 0.998$.

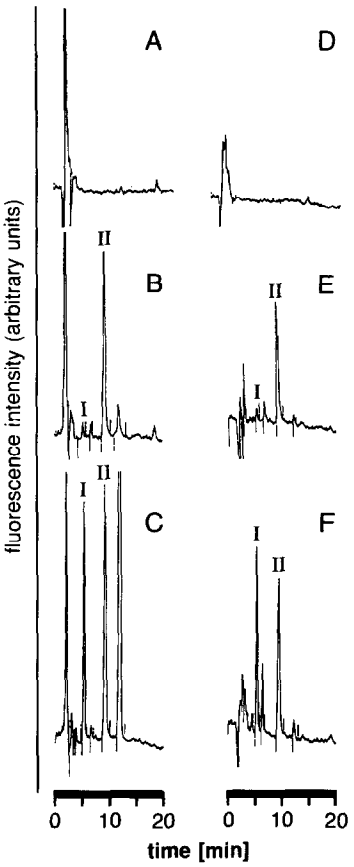

Figure 1
Chromatograms of the extracts of 1 ml blank plasma (A) and blood (D); 1 ml plasma (B) and blood (E) spiked with 2 ng levoprotiline (I) and 30 ng internal standard (II); 1 ml plasma (C) and blood (F) after i.v. administration of 10 mg levoprotiline to healthy volunteer.

Precision and accuracy

The method was validated by the analysis of spiked plasma and blood samples containing several concentrations. The linearity of the calibration curves was tested with samples containing 0, 2, 5, 10, 15, 25, 40, 50 ng ml^{-1} levoprotiline, respectively. The day-to-day variability was monitored for a period of two months by analysis of spiked samples stored at −20°C. The results of within-day and day-to-day precision and accuracy experiments are presented in Table 1.

Sensitivity

The limit of quantitation defined by the relative standard deviation (RSD $\leq$ 10%) was 3 ng ml^{-1}; the limit of detection (RSD $\leq$ 100%) was about 1 ng ml^{-1}. These values are valid for both blood and plasma.

Applicability

The scope of the method was tested by the determination of levoprotiline in blood and plasma from young healthy volunteers treated with single i.v. doses of 2, 5, 10 and 15 mg. Typical blood and plasma profiles are shown in Fig. 2. Since the *R*(−) and S(+) enantiomers are not converted to each other in man [9], the concentrations measured by the presented enantiomeric non-specific method can be assumed as that of levoprotiline.

Table 1

Blood			Plasma		
given [ng ml^{-1}]	mean found [ng ml^{-1}]	RSD [%]	given [ng ml^{-1}]	mean found [ng ml^{-1}]	RSD [%]
Within-day precision and accuracy					
3	3.0	3.7	3	3.1	2.7
4	4.1	5.8	5	4.6	5.2
7	7.2	4.0	10	10.5	8.4
12	12.7	6.6	29	29.1	3.1
25	26.1	4.3	37	36.1	4.6
55	57.0	6.7	50	48.6	4.3
Day-to-day precision and accuracy					
3	2.8	4.5	2	2.0	13.7
12	12.5	6.0	5	4.1	4.9
25	26.2	2.3	10	10.1	7.5
55	55.2	6.1	15	15.1	8.8
68	68.0	1.8	25	23.7	5.4
			50	50.9	1.9

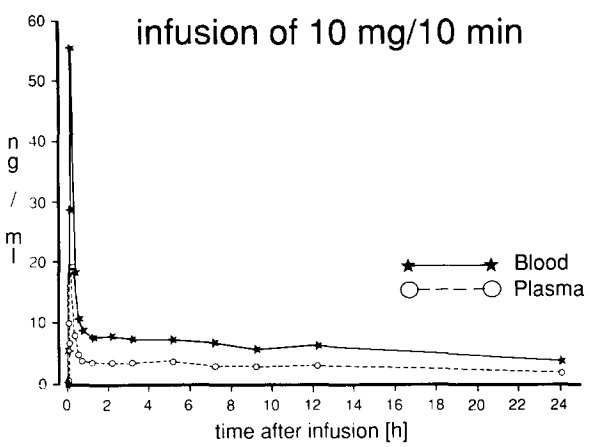

Figure 2
Blood and plasma profiles of levoprotiline in healthy subject after infusion of 10 mg 10 min^{-1}.

Conclusions

The method developed for the routine analysis of low concentrations of unchanged levoprotiline in blood and plasma is shown to be precise and sensitive.

The accuracy and reproducibility of the assay are comparable with those of published methods [5–7]. Yet, the proposed procedure is simpler and less time-consuming, since no sample derivatization is necessary.

The method is suitable for use in bioavailability and pharmacokinetic studies.

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