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Note

Determination of amitriptylinoxide and its major metabolites amitriptyline and nortriptyline in plasma by high-performance liquid chromatography

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Amitriptylinoxide (Equilibrin®) is a tricyclic antidepressant used therapeutically in several European countries. Clinical studies [1—3] and the use of this compound over several years have shown it to have a reduced incidence of adverse side-effects, while still retaining potent antidepressant activity. The underlying mechanism for these properties seems to be a consequence of its pharmacokinetic characteristics. As has been demonstrated in rats [4] and humans [5], the main metabolites of amitriptylinoxide are amitriptyline and, to a much lesser extent, nortriptyline. However, ingestion of amitriptylinoxide leads to lower plasma levels of these metabolites in the peripheral compartment compared with plasma levels of amitriptyline, for example, after direct application of amitriptyline [4, 5]. Furthermore, amitriptylinoxide has been shown to possess very low affinity for those receptor populations [6] that are responsible for the adverse side-effects that are prominent with tricyclic antidepressants. However, it has been demonstrated that amitriptylinoxide leads to a very stable amitriptyline level in rat brain [4], and this might explain the potent antidepressant properties. To characterize the properties of amitriptylinoxide additional pharmacokinetic studies are in progress. Up to now only one method for the simultaneous estimation of amitriptylinoxide, amitriptyline and nortriptyline in human plasma by high-performance liquid chromatography (HPLC) has been published [7]. The extraction procedure described there is quite complicated and the HPLC analysis requires two different runs.

The present paper describes an alternative liquid chromatographic assay, which can be performed both more rapidly and more simply.

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EXPERIMENTAL

Reagents

All reagents were of analytical-reagent grade. They included methanol (p.a., Riedel-de Haën, Hannover, F.R.G.), acetonitrile (HPLC grade S, Rathburn Chemicals, Walkerburn, U.K.), dichloromethane (Baker resi-analyzed, J.T. Baker, Deventer, The Netherlands) and sodium lauryl sulphate (p.a., Fluka, Buchs, Switzerland).

Aqueous solutions were prepared in double-distilled water. Amitriptylin-oxide dihydrate (AMINO) was obtained from Nattermann (Cologne, F.R.G.). Amitriptyline hydrochloride (AMI) was purchased from Hoffmann-La Roche (Basel, Switzerland) and nortriptyline hydrochloride (NOR) from Tropon (Cologne, F.R.G.). Desipramine, the internal standard, was delivered by Ciba-Geigy (Basel, Switzerland).

Sample preparation

Aliquots (1.5 ml) of citrated plasma were added to glass extraction tubes. The internal standard (25 μl of water containing 1.2 μg of desipramine hydrochloride), 1 *M* sodium hydroxide (100 μl) and phosphate buffer (300 μl; pH 12.0) were added and briefly vortexed. The extraction was carried out by shaking the samples for 5 min with dichloromethane (3 × 3 ml). The organic phases were combined and evaporated to dryness. The residue was reconstituted in methanol (80 μl), and an aliquot (25 μl) was injected into the HPLC system and analysed.

Chromatography

The HPLC separations were carried out using a Hewlett-Packard high-performance liquid chromatograph, Model 1090, equipped with a Spectra-Physics integrator, Type Autolab 1. The analytical column was a LiChrosorb CN (Merck, Darmstadt, F.R.G.), particle size 10 μm (250 mm × 4 mm I.D.). The mobile phase, acetonitrile—0.07% ammonia and 0.1% sodium lauryl sulphate in water (85:15) was degassed prior to use. Chromatography was performed at 45°C and a flow-rate of 1.0 ml/min. The eluent was monitored at 210 nm (UV filter detector).

Preparation of the calibration curve

Standard curves were prepared by adding AMINO (20—1000 μg/l), AMI (20—1000 μg/l) and NOR (20—800 μg/l) to drug-free samples of fresh plasma, and processing these standards according to the assay procedure. The ratios of the peak heights of AMINO, AMI and NOR to that of desipramine (internal standard) were used to construct a calibration graph.

RESULTS AND DISCUSSION

The chromatograms of blank plasma and a plasma sample spiked with 400 μg/l AMINO, AMI and NOR and 800 μg/l desipramine as internal standard are shown in Fig. 1. No significant interference was observed at the retention times of the four compounds. Desipramine proved to be very suitable as an internal

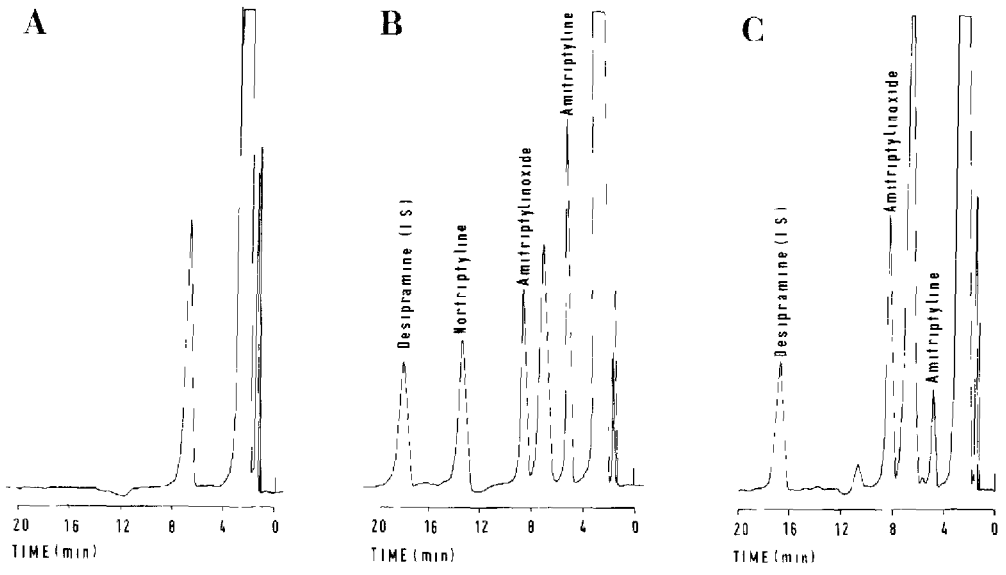

Fig. 1. (A) Chromatogram of blank human plasma. (B) Chromatogram of human plasma containing 400 μg/l amitriptyline, amitriptylinoxide and nortriptyline and 800 μg/l desipramine as internal standard (I.S.). (C) Chromatogram of plasma obtained from a dog 30 min after administration of 2 mg/kg amitriptylinoxide as an intravenous bolus injection.

TABLE I

PRECISION OF THE METHOD (n = 8)

Compound	Concentration (μg/l)	Coefficient of variation (%)
Amitriptylinoxide	20	5.6
	70	3.1
	200	2.9
	500	3.3
	1000	0.4
Amitriptyline	20	9.1
	70	3.2
	200	4.3
	500	2.3
	1000	0.1
Nortriptyline	20	8.7
	70	5.6
	200	2 3
	800	2.2

standard, having similar extraction properties and chromatographic characteristics to AMINO, AMI and NOR.

The calibration curves of peak-height ratios were linear in the added concentration range. The best-fit lines were obtained using linear regression analyses. The results for AMINO, AMI and NOR were: $y = 0.0035x + 0.02$ ($r = 0.999$;

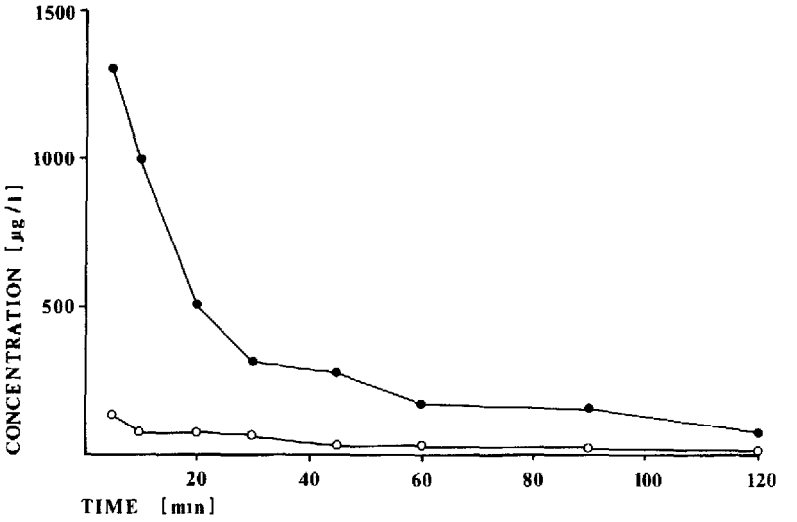

Fig. 2. Concentration of amitriptylinoxide (•) and amitriptyline (○) in dog plasma as a function of time after an intravenous dose of 2 mg/kg.

concentration range 20—1000 µg/l), $y = 0.0060x + 0.06$ ($r = 0.998$; concentration range: 20—1000 µg/l) and $y = 0.0029x + 0.02$ ($r = 0.996$; concentration range: 20—800 µg/l), respectively.

The coefficient of variation (C.V.) for slopes and intercepts of the standard curves was less than 4.0% for all compounds over several determinations ($n = 4$) at different days.

The intra-assay C.V. obtained from replicate analysis ($n = 8$) ranged from 5.6 to 0.4% for AMINO, from 9.1 to 0.1% for AMI and from 8.7 to 2.2% for NOR (Table I).

The recoveries for AMINO, AMI, and NOR as well as desipramine from plasma were in the range 80—100%.

The limit of quantitation was 10 µg/l plasma.

Application of the method was demonstrated by measuring the plasma AMINO and AMI levels in a beagle dog following intravenous bolus administration of a 2.0 mg/kg AMINO dose. Fig. 2 shows the plasma AMINO and AMI concentrations versus time plots from this experiment. The plasma levels of NOR were below the estimation level.

CONCLUSIONS

The assay described here provides a faster extraction procedure than the previously published method [7] and requires only one HPLC analysis for the detection of amitriptylinoxide and its major metabolites.

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