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Note

High-performance liquid chromatographic determination of cimoxatone and its O-demethyl metabolite in plasma

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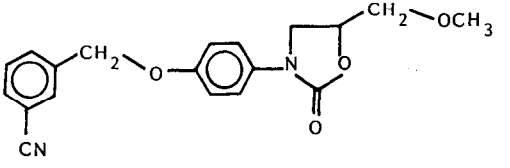
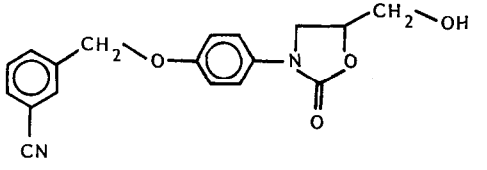
Cimoxatone (I) (MD 780515, 3-[4-(3-cyanophenylmethoxy)phenyl]-5-(methoxymethyl)-2-oxazolidinone, Fig. 1), is a new, selective and reversible inhibitor of type A monoamine oxidase (MAO) both in rat [1, 2] and man [3]. Cimoxatone is extensively metabolized in man [4] and its major plasma metabolite, MD 770222 (II) {3-[4-(3-cyanophenylmethoxy)phenyl]-5(hydroxymethyl)-2-oxazolidinone}, is also a selective and reversible type A MAO inhibitor, but 7—8 times less inhibitory than the parent compound [5].

(I)



(II)



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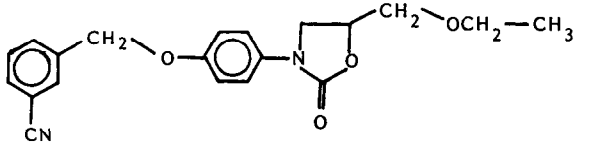

Fig. 1. Structures of cimoxatone (I), the O-demethyl metabolite, MD 770222 (II) and the internal standard (III).

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A high-performance liquid chromatogrpahic (HPLC) method was previously developed in our laboratory using a column packed with silica gel, whose limit of sensitivity was 0.03 mg/l for both compounds and which required a 5—6 ml plasma sample. The measurement of I and II in plasma requires a more sensitive analytical procedure, thus a technique using reversed-phase HPLC with automatic injection was developed and is described in this paper.

MATERIALS AND METHODS

Chemicals and solvents

Pure standards of I, II and MD 780918 (III) {3-[4-(3-cyanophenylmethoxy)-phenyl]-5(ethoxymethyl)-2-oxazolidinone} were synthesized in the Department of Organic Chemistry at Delalande Research Centre; III was used as an internal reference standard.

A solution of water—acetonitrile (90:10, v/v) containing 5 mg/l of III (0.5 mg in 100 ml) was prepared.

All the solvents and chemicals were of analytical grade: toluene, *n*-heptane, acetonitrile (SDS), methanol (Carlo Erba), potassium dihydrogen phosphate and phosphoric acid (Merck).

Extraction procedure

A 100-μl volume of the internal standard solution and 1 ml of plasma were added to a conical tapered tube. After mixing on a Vortex, 5 ml of toluene were added. Samples were extracted on a mechanical shaker (Luckham) for 30 min and the two phases were then separated by centrifugation at 4°C (1000 *g* for 10 min). The organic phase was transferred into a second tube and evaporated to dryness under nitrogen at 40°C. The dry extract was dissolved in 500 μl of *n*-heptane; the solution was shaken on a Vortex mixer, then 500 μl of water—acetonitrile (90:10, v/v) were added. The biphasic solution was vigorously shaken on a Vortex mixer for 45 sec and after centrifugation (5 min at 1000 *g*) the aqueous phase (470 μl) was transferred into a vial, the volume was adjusted to 850 μl with water and injected onto the HPLC column. Automatic injection of samples was carried out every 20 min.

High-performance liquid chromatography

Analyses were carried out with a Micromeritics 750 liquid chromatograph equipped with a 786 UV—visible spectrophotometer and a 725 automatic injector (500-μl injection loop). The ultraviolet (UV) detector was set at 240 nm which corresponds to a relative maximum of absorption for cimoxatone. The UV signal (0.005 a.u.f.s.) was recorded on a 3390A Hewlett-Packard integrator. A stainless-steel column was packed at 400 bar (isopropanol) with 5 μm ODS Spherisorb (batch 18/109). Methanol—phosphate buffer (55:45, v/v) was used as a mobile phase (1 ml/min at 20°C). The buffer was prepared from 0.05 m*M* KH_2PO_4 and the pH adjusted to 4 with phosphoric acid.

Calibration curves

A 1 mg amount of both I and II was dissolved in 100 ml of a solution of water—acetonitrile (90:10, v/v). The solution containing 10 mg/l of the two

compounds (solution A) was then diluted to 1 mg/l (solution B) and to 0.1 mg/l (solution C). The calibration curve was prepared with solution C (0.01, 0.02 and 0.05 mg/l), solution B (0.1 and 0.5 mg/l) and solution A (1 mg/l). The aqueous solutions of the standards were stored at 4°C and renewed every month; no degradation of I, II and the internal standard occurred during this period.

The samples were then processed according to the extraction procedure and to the HPLC sections.

RESULTS AND DISCUSSION

Extraction of I and II from plasma was assayed with toluene, diethyl ether, chloroform and ethyl acetate. A single extraction with toluene provided the cleanest extract. A further purification of the plasma extracts was obtained by dissolution in *n*-heptane followed by extraction with water—acetonitrile (90:10, v/v) which was then injected into the HPLC system.

As expected from their chemical structures, pH changes did not affect the recovery of I and II which, with toluene, was near 90% for both compounds. The extraction of I and II at alkaline pH (>12) led to the degradation of both compounds. In fact, 2-oxazolidinones are not stable at alkaline pH and are hydrolysed to the corresponding β-amino-alcohol derivatives [6]. The extraction from plasma was carried out at pH 7.4.

Plasma extracts from healthy adult volunteers after a single oral dose of the drug showed that I, II and the internal standard were well separated using an ODS Spherisorb reversed-phase column with acetonitrile—0.05 m*M* phosphate buffer pH 5.5 (45:55, v/v) as a mobile phase. No interference occurred from any endogenous compound. However, when the method was applied to monitor plasma concentrations of I and II in depressed patients, it was found that oxazepam interfered with the analysis of the metabolite. Anxiolytics and hypnotics are currently co-administered during chronic treatment with antidepressant drugs. The chromatographic conditions were therefore modified and the mobile phase was replaced by methanol—0.05 m*M* phosphate buffer pH 4.0 (55:45, v/v). Under these conditions, oxazepam, diazepam, demethyl diazepam, lorazepam, chlordiazepoxide and flunitrazepam did not interfere with the analysis of I, II and III. Chromatograms of plasma extracts from a depressed patient treated with I and lorazepam are shown in Fig. 2.

The calibration curves $Y = aX + b$ were drawn by least-squares linear regression analysis of spiked plasma concentration of I or II (X) versus the peak height ratio (Y) of I(II)/internal standard. One sample was prepared for each concentration. A linear response was observed in a range of concentrations between 0.01 and 1.0 mg/l for both compounds. The limit of detection was 0.005 mg/l for both I and II. The results from 16 calibration curves performed during a three-months period showed correlation coefficients (r^2) always > 0.999; the slope of the calibration curves ranged from 3.321 to 4.044 for I and from 5.304 to 7.186 for II. If compared with the results obtained from the grouped data (Table I) the variability of the slope for each single calibration curve exceeded the 95% confidence interval for both compounds. These day-to-day variations were attributed to the extraction procedure and/or to

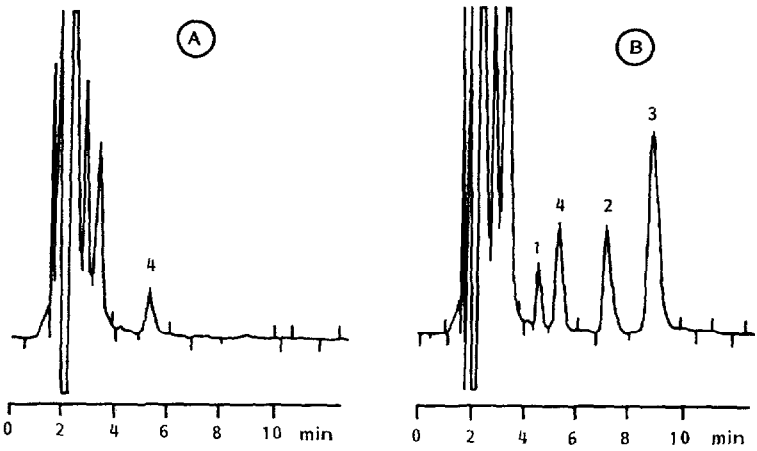

Fig. 2. HPLC chromatograms of plasma extracts obtained from a depressed patient before drug treatment (A) and 24 h after the first 40-mg dose (B). Peaks: 1 = II (0.064 mg/l); 2 = I (0.176 mg/l); 3 = internal standard (0.5 mg/l); 4 = lorazepam.

TABLE I

ACCURACY AND PRECISION OF GROUPED DATA

Cimoxatone (I)				MD 770222 (II)			
Spiked conc. (mg/l)	Found conc. (mg/l)	S.D./X (%)	No. of measurements	Spiked conc. (mg/l)	Found conc. (mg/l)	S.D./X (%)	No. of measurements
0.010	0.009	11	14	0.010	0.011	9	14
0.020	0.020	10	13	0.020	0.021	10	13
0.050	0.050	4	16	0.050	0.051	4	16
0.100	0.098	4	16	0.100	0.097	2	16
0.500	0.508	2	15	0.500	0.501	2	15
1.000	0.996	1	16	1.000	1.000	1	14

Linear least-squares regression analysis (grouped data)

	Equation ($Y = aX + b$)	Slope (a) 95% confidence intervals	Intercept (b) 95% confidence intervals
I	$Y = 3.558X + 0.0045$	3.504/3.612	−0.0216/0.0305
II	$Y = 5.978X$ 0.0051	5.817/6.138	−0.0768/0.0667

the chromatographic analysis, and showed that it is preferable to repeat a calibration curve for each set of samples rather than use the mean calibration curve which gives only the accuracy and the precision of the grouped data. The reproducibility of the technique is also confirmed by the linear relationship between the slope values of the single calibration curves for I and II ($r^2 = 0.935, p < 0.001$).

Plasma concentrations of I and/or II may occasionally exceed 1 mg/l at the doses used in clinical studies, but it was demonstrated that the calibration curve is linear up to 1.5 mg/l for each compound. An example of the plasma concentration—time curve determined in one healthy adult after a single oral dose of a 40-mg tablet of I is given in Fig. 3.

In conclusion, the full automatic procedure makes the technique suitable for routine analyses. This simple and sensitive analytical method has been ex-

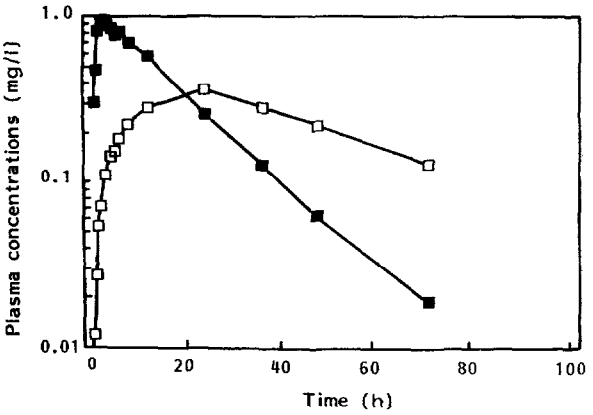

Fig. 3. Plasma concentrations of I (■) and II (□) from one subject after oral administration of the drug (40-mg tablet).

tensively used for the investigation of the pharmacokinetics of I and its major plasma metabolite II, as well as for plasma monitoring in depressed patients following repeated dose treatment.

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