Journal of Chromatography, 495 (1989) 324–329
Biomedical Applications
Elsevier Science Publishers B V Amsterdam — Printed in The Netherlands

CHROMBIO 4915

Note

Automated high-performance liquid chromatographic assay for the β-carboline derivative Abecarnil in plasma

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(First received February 7th, 1989 revised manuscript received June 7th, 1989)

Abecarnil (isopropyl-6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate, I, ZK 112 119) is a newly synthesized β-carboline derivative It has been reported to exhibit anxiolytic and anticonvulsant activity in a number of animal models Abecarnil is a partial agonist at the benzodiazepine receptor antagonizing ataxia produced by full agonists such as benzodiazepines. The potency of the new compound in displacing benzodiazepines from the receptor is approximately five times that of diazepam [1]

The objective of the present study was to develop a fully automated high-performance liquid chromatographic (HPLC) procedure for the determination of Abecarnil in plasma as an alternative to a method using diethyl ether extraction, which has recently been described in the literature [2] This new technique was applied to plasma samples obtained in a pharmacokinetic investigation in rabbits

EXPERIMENTAL

Animal study

Five female rabbits (New Zealand, white, 3–4 kg) received an intragastric dose of 100 mg/kg Abecarnil administered as a microcrystalline suspension in Klucel LF–Myrj 53–physiological saline at a concentration of 10 mg/ml Before and 0 5, 1, 1 5, 2, 4, 6, 8 10, 24, 36 and 48 h after drug treatment, blood was collected from an ear vein and stabilized immediately by the addition of sodium fluoride (40 mmol in blood) The plasma was stored frozen until analysed

Chemicals

Methanol and ammonium carbonate were of analytical-reagent grade (Merck, Darmstadt, F R G) Water was distilled twice in an all-glass apparatus prior to use

Chromatographic assay procedure

The HPLC system consisted of two 6000 A solvent-delivery pumps (Waters, Konigstein, F R G), a WISP autosampler (Waters), an SE-2 column-switching module (Gynkotek, Munich, F R G) and an HP 1046 A fluorescence detector (Hewlett-Packard), set at excitation and emission wavelengths of 279 and 427 nm, respectively The chromatographic columns used were Spherisorb ODS II 125 mm×4 6 mm I D (3 μm particle size) for the main column and 40 mm×4 6 mm I D (5 μm particle size) for each of the two guard columns. The mobile phase was either water (pump A) or methanol–0 01 *M* aqueous ammonium carbonate solution (pump B) (80 20 or 70 30, v/v) The set-up of the HPLC system, which was operated at ambient temperature (20°C), was as described previously [3]

Plasma samples were directly injected by the autosampler into a guard column using water at a flow-rate of 1 ml/min as mobile phase During a 4-min wash cycle, salts and proteins were eluted from the guard column, whereas Abecarnil was retained Thereafter, two valves were switched allowing pump B to elute the drug from the guard column onto the main column with subsequent separation from sample constituents and measurement in the fluorescence detector

During the analysis cycle the second guard column was equilibrated with water to be ready for loading with the next sample This alternate washing and loading of two guard columns allowed for a high sample throughput The run time of each individual cycle was either 15 min (80%, v/v, methanol in the mobile phase) or 30 min (70%, v/v, methanol) The retention time of Abecarnil on the main column was 4 5 and 16 5 min, respectively

Calibration

For calibration, standard curves were constructed by spiking drug-free plasma with Abecarnil at concentrations of 0–400 ng/ml and injecting 0 2-ml samples Detector signals were transferred on-line to a mainframe computer (Honeywell CHB), where peak areas were calculated Calibration curves were obtained by linear regression analysis

RESULTS

Linearity

In the concentration range tested (0–400 ng/ml) calibration curves were linear with a mean regression coefficient of 0 996 ± 0 004

Mean equations obtained from an intra-assay ($n=5$) and inter-assay ($n=4$) comparison were $y=325(\pm297)+433(\pm38)x$ and $y=215(\pm139)+486(\pm59)x$, respectively

TABLE I

PRECISION AND ACCURACY OF ABECARNIL DETERMINATION BY AN AUTOMATED HPLC ASSAY

Calculations were performed using calibration curve No 3 (within-one-day experiment) as standard

Concentration (ng/ml)	Precision[a]		Accuracy[b]	
	Intra-assay	Inter-assay	Intra-assay	Inter-assay
2 5	13 5	18 5	148 ± 20	130 ± 24
5	4 4	12 8	113 ± 5	117 ± 15
12 5	6 4	11 9	93 ± 6	111 ± 13
25	9 6	16 8	98 ± 9	106 ± 18
50	6 3	11 9	103 ± 7	118 ± 14
125	10 0	18 6	93 ± 9	119 ± 22
250	7 8	11 1	117 ± 9	126 ± 14

[a]Coefficient of variation (%) $n = 4$
[b]Percentage of nominal concentration

Precision

At 5–250 ng/ml, the mean intra- and inter-assay precisions were 7 4 and 13 9%, respectively Details are summarized in Table I

Accuracy

The mean intra- and inter-assay accuracy at 5–250 ng/ml was 103 ± 10 and 116 ± 7% (Table I)

Sensitivity

The limit of detection in plasma at a signal-to-noise ratio of 2 was 0 2 ng/ml

Selectivity

No interference was observed from endogenous substances or from contamination from tubes or containers (Fig 1)

Animal study

After intragastric administration of 100 mg/kg, peak plasma levels of 440 ± 223 ng/ml were reached after 24 ± 9 h (Table II, Fig 2). The high dose prolonged the absorption process and resulted in either several peaks and minima or in plateaus with late increases in plasma levels

The terminal half-life ($t_{1/2}$) calculated individually from the data of three animals was 6 7 ± 0 5 h From the mean-values curve, $t_{1/2} = 9.8$ h was obtained

The area under the plasma level time curve (AUC) from zero to the last sampling point was 8 85 ± 3 81 h μg/ml The bioavailability, calculated from

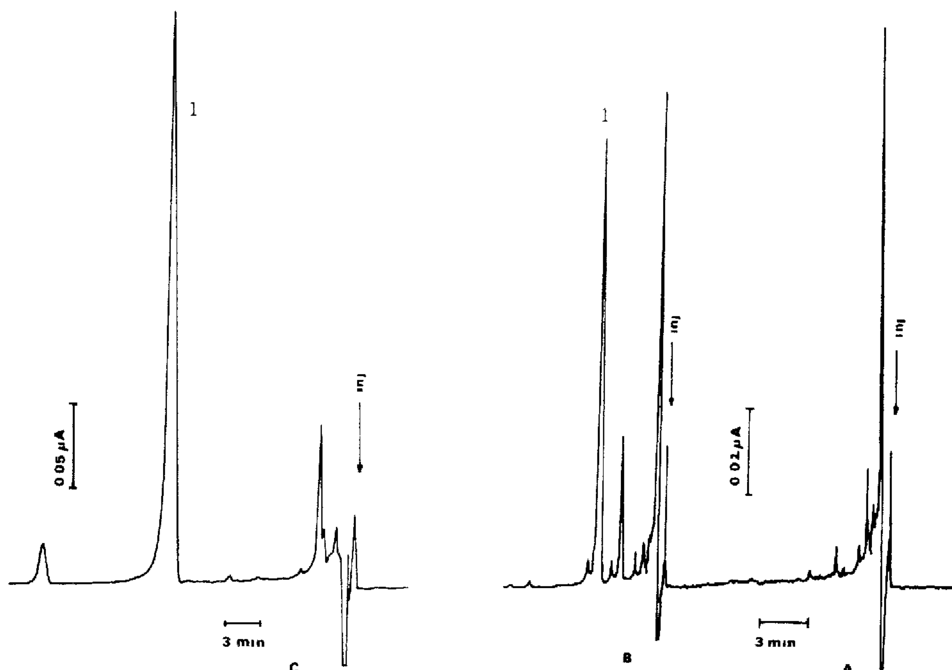

Fig 1 Chromatograms of Abecarnil (peak 1) in human drug-free plasma spiked with 0 (A) or 5 ng per 0 2 ml (B), and in rabbit plasma (animal No 3) obtained 1 h after intragastric administration of 100 mg/kg (C) The mobile phase for A and B was 80% (v/v) methanol (retention time of Abecarnil, 4 5 min), for C it was 70% (v/v) methanol (retention time, 16 5 min)

TABLE II

PHARMACOKINETIC PARAMETERS OF ABECARNIL IN FIVE FEMALE RABBITS (1-5) AFTER INTRAGASTRIC ADMINISTRATION OF 100 mg/kg

Parameter[a]	1	2	3	4	5	Mean ± S D
C_{max} (ng/ml)	157	259	606	507	673	440 ±223
t_{max} (h)	24	36	24	24	10	24 ±9
$t_{1/2}$ (abs) (h)	-	-	4 9	-	2 2	3 6 (n=2)
$t_{1/2}$ (h)	-	-	6 7	7 1	6 2	6 7 ±0 5
AUC_{0-n} (h μg/ml)	5 31	5 04	13 9	8 78	11 2	8 85±3 81

[a] C_{max} = peak plasma level, t_{max} = time to reach peak level, $t_{1/2}$(abs) = half-life of absorption, $t_{1/2}$ = half-life of disposition, AUC_{0-n} = area under the plasma level-time curve from zero to the last time point

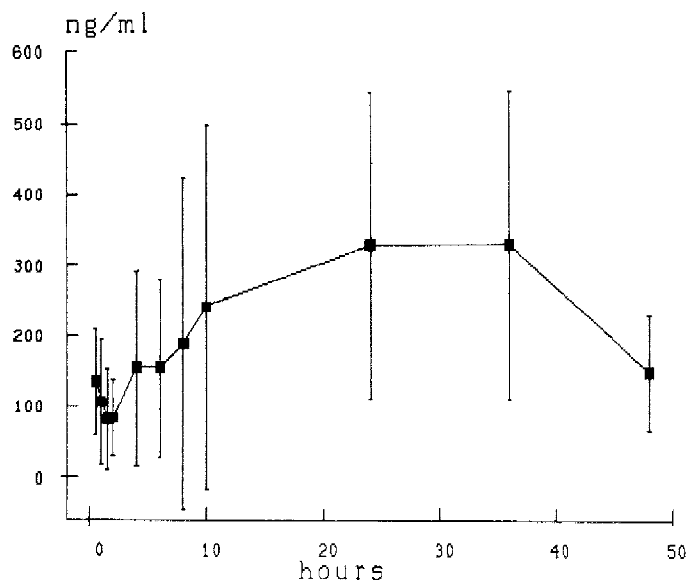

Fig 2 Plasma levels (mean ± S D) of Abecarnil in five female rabbits after intragastric administration of 100 mg/kg

this AUC and from an AUC_{iv} determined previously in a different group of animals (26 4 h ng/ml, 0 1 mg/kg), was 34%

DISCUSSION

The aim of this study was to apply the column-switching technique, which had originally been described by Roth et al [4], to the determination of the β-carboline derivative Abecarnil in plasma The method developed earlier [2] used diethyl ether extraction prior to HPLC separation The advantage of the column-switching technique is the possibility of fully automating the whole analysis procedure without any need for off-line purification The data of the present study show that Abecarnil can be determined in plasma by the column-switching technique with accuracy and precision similar to that of conventional extraction methods Additionally, the limit of detection is the same after both assay procedures

The new technique was applied to the determination of Abecarnil plasma concentrations in rabbits treated intragastrically with 100 mg/kg Abecarnil The drug was slowly absorbed, reaching maximum levels of 440 ± 223 ng/ml after 24 h Thereafter concentrations declined with a terminal half-life of 6 7 h Urine samples were not measured, because in earlier studies with a number of different species including rat, rabbit, dog, monkey and also humans the unchanged drug could not be detected in urine [2]

The new method is not applicable either to the determination of Abecarnil in faeces samples, which still have to be extracted for the measurement of unabsorbed drug, and or to the analysis of Abecarnil in tissue samples

The column-switching technique therefore seems to be a suitable alternative to the conventional method for the determination of Abecarnil in plasma samples.

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