ELSEVIER

Journal of Chromatography B, 700 (1997) 209–213

JOURNAL OF
CHROMATOGRAPHY B

Determination of 6,4′-bis-(2-imidazolinylhydrazone)-2-phenylimidazo[1,2-*a*]pyridine in plasma and whole blood by high-performance liquid chromatography

T.S. Selvamuniandy[a], N. Mohamed[a,b], S.M. Mansor[a], N.K. Nair[a], V. Navaratnam[a,*]

[a]*Centre for Drug Research, Universiti Sains Malaysia, 11800 Penang, Malaysia*
[b]*School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia*

Received 2 July 1996; revised 1 April 1997; accepted 14 April 1997

Abstract

A selective and sensitive HPLC assay for the quantitative determination of a new antifilarial drug, 6,4′-bis-(2-imidazolinylhydrazone)-2-phenylimidazo[1,2-*a*]pyridine (CDR 101) is described. After extraction from plasma and blood, CDR 101 was analysed using a C_{18} Nucleosil ODS column (250×4.6 mm, 5 μm particle size) and mobile phase of acetonitrile–0.05 *M* ammonium acetate adjusted to pH 3.0, with UV detection at 318 nm. The mean recoveries of CDR 101 in plasma and blood over a concentration range of 25–500 ng/ml were 95.5±2.01% and 83.3±1.87%, respectively. The within-day and day-to-day coefficient of variations for plasma were 3.23–6.21% and 2.59–9.90%, respectively, those for blood were 2.59–5.92% and 2.89–6.82%, respectively. The minimum detectable concentration for CDR 101 was 1 ng/ml in plasma and 2.5 ng/ml in whole blood. This method was found to be suitable for clinical pharmacokinetic studies.

Keywords: 6-4′-Bis-(2-imidazolinylhydrazone)-2-phenylimidazo[1,2-*a*]pyridine

1. Introduction

Filarial infections are a major cause of disease in many tropical and subtropical countries. The classical drugs, diethylcarbamazine and suramin, have limited efficacy [1]. Currently, ivermectin is being used in the treatment of filariasis although it is only effective against microfilariae [2–4]. Benzimidazole carbamates are known to possess potent antifilarial activity but because of their low bioavailability, the use of these compounds in the treatment of filarial infections is limited [5–7].

The search for new antifilarial drugs for effective and safe treatment of filariasis has led to the synthesis of 6,4′-bis-(2-imidazolinylhydrazone)-2-phenylimidazo[1,2-*a*]pyridine (CDR 101, Fig. 1). This compound was selected from screening under the UNDP/World Bank/WHO Drug Discovery Programme. Apart from basic toxicological and efficacy data, information on the preclinical and biochemical pharmacology of this compound is lacking. In order to establish this information, a reliable assay for CDR 101 in biological fluids is a prerequisite. This paper describes a sensitive, selective and reproduc-

*Corresponding author.

0378-4347/97/$17.00 Copyright © 1997 Elsevier Science B.V. All rights reserved
PII S0378-4347(97)00246-6

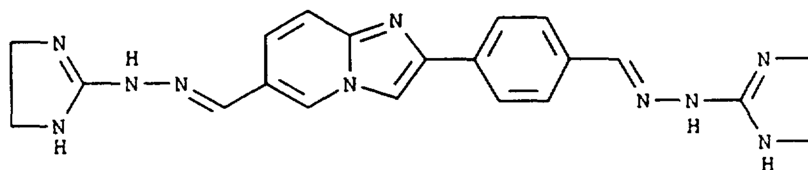

Fig. 1. Structural formula of CDR 101.

ible high-performance liquid chromatographic (HPLC) method for the determination of CDR 101 in plasma and whole blood.

2. Experimental

2.1. Chemicals and reagents

CDR 101 was supplied by Ash Stevens (Detroit Research Park, MI, USA). Mebendazole (MBZ) was obtained from Sigma (St Louis, MO, USA). Acetonitrile was HPLC grade, ammonium acetate and chloroform were analytical grade were purchased from Merck (Darmstadt, Germany). Analytical reagent grade acetic acid and sodium chloride were obtained from BDH (Poole, UK). Other chemicals and solvents used were of analytical grade.

2.2. Chromatographic conditions

Analyses were performed on a HPLC system consisting of a quaternary pump system (HP 1050, Hewlett-Packard, Waldbronn, Germany) equipped with a sample injector (Model 7125, Rheodyne, Cotati, CA, USA) coupled to a variable ultraviolet absorbance detector (Model 486, Waters Associates, Milford, MA, USA) operating at 318 nm. The chromatogram was recorded using an electronic integrator (HP 3392A, Hewlett-Packard, Avondale, PA, USA). Chromatographic separations were carried out using a C_{18} Nucleosil ODS-2 column, 250×4.6 mm I.D., 5 μm particle size (Keystone, Bellafonte, PA, USA). The mobile phase consisted of acetonitrile in channel A and 0.05 *M* ammonium acetate buffer, adjusted to pH 3.00 with acetic acid in channel B. The flow-rate was kept constant at 1.0 ml/min.

Optimum analysis of the peak of interest and the internal standard necessitated a gradient elution which commenced with channel A supplying 18% of the mobile phase. A linear gradient began at 8 min which brought channel A up to 58% at 11 min and this condition was maintained up to 16 min. At 18 min the gradient was brought to its initial condition and was held for 5 min for equilibration to occur before the next analysis was begun.

2.3. Extraction procedure

The extraction was performed in glass culture tubes (10 ml capacity) pretreated with dichlorodimethylsilane to 5% (v/v) toluene in order to minimise drug adsorption. To samples of plasma (1.0 ml) or whole blood (200 μl diluted to 400 μl with normal saline) containing the internal standard, MBZ (75 ng; 50 μl) and CDR 101 in various concentrations was added methanol (1.5 ml) to precipitate plasma–blood protein. Following vortex-mixing (30 s), the samples were centrifuged at 1000 *g* for 10 min before being transferred to clean culture tubes to which borate buffer of pH 9.5 (1.0 ml) was added. This mixture was extracted with 8 ml chloroform by vortex-mixing for 1.5 min. After further centrifugation at 1000 *g* for 10 min, the aqueous phase was discarded and the organic layer transferred to a clean tube and evaporated to dryness under a gentle stream of nitrogen at room temperature (25°C). The dry residue was reconstituted in methanol (50 μl) and 20 μl was injected into the liquid chromatograph.

2.4. Calibration

The calibration curve was prepared by spiking drug-free plasma and blood samples with standard solutions of CDR 101 and a fixed amount of internal standard (75 ng; 50 μl) to give a concentration range of 12.5–500 ng/ml. The samples were extracted as described in Section 2.3 and the peak heights of the drug were plotted against the corresponding concentrations of the drug. Linear regression of the peak

height ratio versus the drug concentration was performed in order to estimate the slope, intercept (peak height for zero concentration) and correlation coefficient of the curve.

2.5. Recovery, within-day and day-to-day precision

The recovery of the extraction procedure for CDR 101 was determined by comparing the peak height from extracted spiked plasma or blood samples with the peak height of the standard solution of the corresponding concentrations. The within-day precision was assessed by 5 replicate assays of 4 samples each, both with plasma and blood. The day-to-day assay variation was evaluated over a period of 5 days, with 4 samples each, both with plasma and blood. A 6-point calibration curve and two standard samples (blind) were part of each assay run.

2.6. Animal study

In order to test the ability of the method to measure CDR 101 in the course of pharmacokinetic studies, CDR was administered intravenously to a male Wistar rat at a dose of 400 μg/kg. Blood samples (200 μl) were collected from the carotid artery predose and at 15, 30, 60, 75, 90, 120, 150, 180, 210 and 240 min after drug administration. An equivalent volume of saline was infused to replaced blood lost in sampling. The samples were stored at −20°C until analysis. The samples were analysed in one batch with 5 calibration samples and 2 standard samples.

2.7. Pharmacokinetic analysis

The area under the blood concentration–time curve (AUC) was calculated by the linear trapezoidal rule. Other pharmacokinetic parameters were calculated using standard model-independent formulae [8]. Maximum concentration (C_{max}) and time to reach maximum concentration (T_{max})are observed values.

3. Results and discussion

The extraction method employed produced extremely clean chromatograms. The HPLC method provided base line separation of the components of interest in a reasonable analysis time. Fig. 2 illustrates the chromatograms of (a) the standard mixture of CDR 101 and MBZ, (b) extracted blank plasma and (c) extracted CDR 101 and MBZ from plasma. Fig. 3 shows the chromatograms of CDR 101 extracted from whole blood of rat receiving 400 μg/kg CDR 101 intravenously. There was no chromatographic interference from any endogenous compounds. The retention time for CDR 101 and MBZ were 10.74 min and 15.86 min, respectively. Plasma and whole blood samples of rats dosed with CDR 101 via the oral and i.v. route did not show the

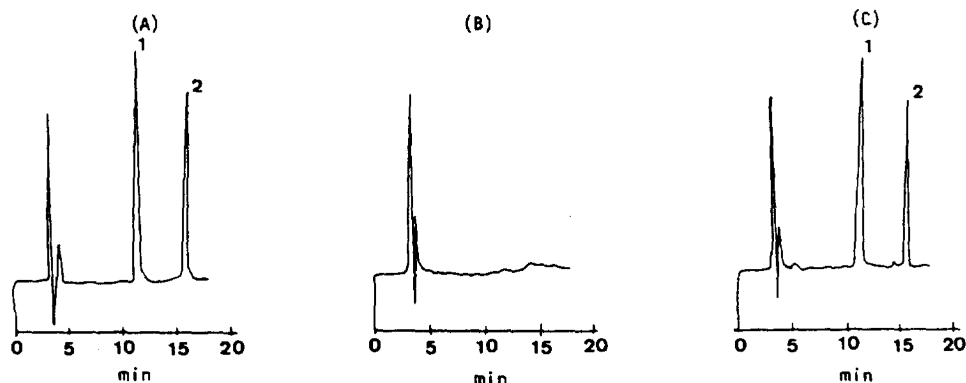

Fig. 2. Representative chromatograms of (A) a standard mixture of CDR 101 (100 ng) and MBZ (30 ng) in methanol, (B) extracted blank plasma and (C) extracted CDR 101 (250 ng/ml) and MBZ (75 ng/ml) from plasma. Peaks: 1=CDR 101; 2=MBZ.

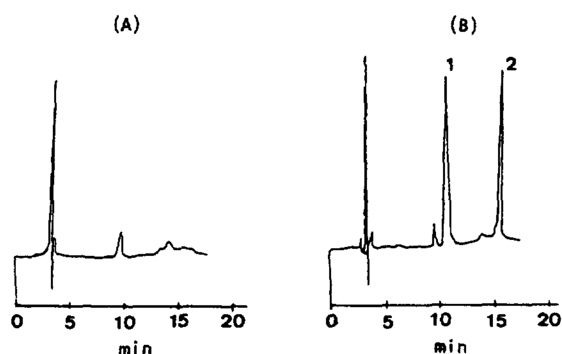

Fig. 3. Chromatograms of CDR 101 in whole blood obtained from rat study following i.v. administration of 400 μg/kg CDR 101, (A) at predose and (B) at 15 min after dosing. Peaks: 1=CDR 101; 2=MBZ.

presence of a metabolite. This was confirmed by IR spectrometry.

The calibration curves were linear for plasma as well as for whole blood over the concentration range 12.5–500 ng/ml. Linear regression analysis followed the least square procedure, resulting in the equation $y=bx+a$ where a denotes the intercept and b the slope. It yielded the correlation coefficient $r>0.999$. The equation of the calibration plots ($n=6$) were $y=0.0016x-0.0166$ for plasma and $y=0.0019x+0.0168$ for whole blood.

The recoveries for CDR 101 in plasma and whole blood are summarised in Table 1. The recovery of CDR 101 in blood appeared to be slightly less than that of plasma. Mean recoveries for CDR 101 in plasma and blood were 95.51±2.01% and 83.35±1.87%, respectively. The within-day and day-to-day variations were ranged from 2.59% to 9.90% (Tables 2 and 3). The limit of detection for this assay was 1 ng/ml for plasma and 2.5 ng/ml for whole blood.

For practical validation the assay method was applied to blood samples obtained from a rat given 400 μg/kg CDR 101 intravenously. The blood-concentration–time profile of CDR 101 is shown in Fig. 4. Following intravenous administration in a rat, CDR 101 is eliminated from blood with half-life, clearance and volume of distribution of 102.36 min, 200.26 ml/min kg and 18.42 l/kg, respectively. The area under the blood concentration–time curve (AUC) was 6290 ng min/ml. The maximum concentration (C_{max}) of CDR 101 (233.60 ng/ml) was observed at 15 min post-dose.

In summary, we have reported a sensitive, selective and reproducible HPLC method for determination of CDR 101 in plasma and whole blood. The method has demonstrated to be suitable for phar-

Table 1
Mean recoveries of CDR 101 in plasma and whole blood ($n=5$)

Amount spiked (ng/ml)	Plasma		Whole blood	
	Mean recovery (%)	C.V. (%)	Mean recovery (%)	C.V. (%)
25	94.10	2.59	83.36	3.26
50	93.81	4.18	85.70	2.13
250	95.98	7.92	81.13	6.71
500	98.15	5.92	83.21	5.42

Table 2
Within-day and day-to-day precisions of the method for determination of CDR 101 in plasma

Plasma concentration (ng/ml)	Within-day precision		Day-to-day precision	
	Concentration determined (ng/ml)	C.V. (%)	Concentration determined (ng/ml)	C.V. (%)
25	24.85	3.23	23.65	2.59
50	51.52	6.21	48.21	6.71
250	248.03	2.72	256.30	9.90
500	548.22	5.41	497.15	5.32

$n=5$ for each concentration for within-day precision; one concentration line each for 5 days for day-to-day precision.

Table 3
Within-day and day-to-day precisions of the method for determination of CDR 101 in whole blood

Plasma concentration (ng/ml)	Within-day precision		Day-to-day precision	
	Concentration determined (ng/ml)	C.V. (%)	Concentration determined (ng/ml)	C.V. (%)
25	23.25	5.65	26.25	2.89
50	49.02	2.89	48.09	6.82
250	242.56	5.15	248.37	5.65
500	521.13	5.92	502.33	6.26

$n=5$ for each concentration for within-day precision; one concentration line each for 5 days for day-to-day precision.

macokinetic studies, the more so as preliminary studies have shown substantial absorption after oral dosing.

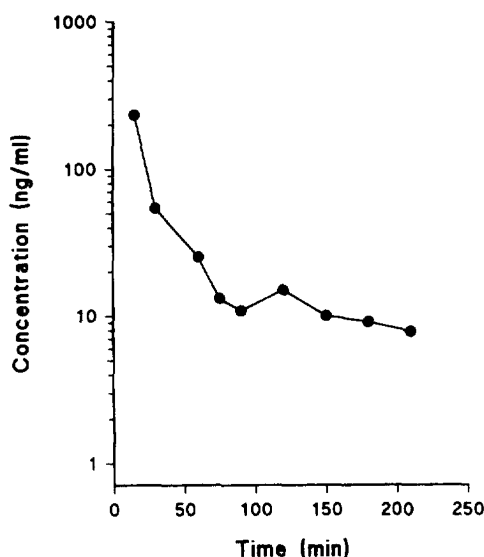

Fig. 4. Blood concentration versus time profile in a rat following i.v. administration of 400 μg/kg CDR 101.

Acknowledgments

The study received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease (TDR). T.S.S. is in receipt of a Postgraduate Studentship from Universiti Sains Malaysia. The authors also wish to acknowledge Prof. W.H. Wernsdorfer for his helpful commends on this manuscript.

References

[1] WHO Technical Report Series, Lymphatic Filariasis, Fourth Report of the WHO Expert Committee on Filariasis, No. 702, 1984.
[2] W.C. Campbell, Ann. Rev. Microbiol. 45 (1991) 445.
[3] H.R. Taylor, M. Pacque, B. Munoz, B.M. Greene, Science. 250 (1990) 116.
[4] V. Kumaraswami, E.A. Ottesen, V. Vijayasekaran, S.U. Devi, M. Swaminathan, J. Am. Med. Assoc. 259 (1988) 3150.
[5] A. Dominguez-Vazquez, H.R. Taylor, B.M. Greene, A.M. Ruvalcaba-Macias, A.R. Rivas-Alcala, R.P. Murphy, F. Beltran-Hernandez, Lancet. i (1983) 1139.
[6] K. Awadzi, M. Hero, O. Opoku, D.W. Buttner, H.M. Gilles. Trop. Med. Parasitol. 42 (1991) 383.
[7] M. Van Hoegaeden, S. Ivanoff, F. Flocard, A. Salle, B. Chaband, Ann. Trop. Med. Parasitol. 81 (1987) 275.
[8] M. Rowland and N.T. Tozer, Clinical Pharmacokinetics. Concept and Applications, Lea and Fiebiger, London, 1989.