Journal of Chromatography, 615 (1993) 303–307
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6800

Determination of a new antifilarial drug, UMF-058, and mebendazole in whole blood by high-performance liquid chromatography

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(First received November 27th, 1991; revised manuscript received February 22nd, 1993)

ABSTRACT

A rapid and selective high-performance liquid chromatographic assay for simultaneous quantitative determination of a new antifilarial drug (UMF-058, I) and mebendazole (MBZ) is described. After a simple extraction from whole blood, both compounds were analysed using a C_{18} Nova Pak reversed-phase column and a mobile phase of methanol–0.05 *M* ammonium dihydrogenphosphate (50:50, v/v) adjusted to pH 4.0, with ultraviolet detection at 291 nm. The average recoveries of I and MBZ over a concentration range of 25–250 ng/ml were 92.0 ± 7.7 and 84.4 ± 4.4%, respectively. The minimum detectable concentrations in whole blood for I and MBZ were 7 and 6 ng/ml, respectively. This method was found to be suitable for pharmacokinetic studies.

INTRODUCTION

Mebendazole (MBZ) is a poorly water-soluble drug widely used for the treatment of intestinal helminth infections. Its limited absorption and rapid metabolism [1] mean that only high and prolonged doses are effective in the treatment of systemic infections [2]. In the case of lymphatic filariasis, high doses are not recommended because they are known to be toxic in large doses and teratogenic in animals. Therefore the drug has not yet been cleared for use in pregnancy [3].

There is an urgent need for new effective and safe antifilarial drugs with high bioavailability. In order to fulfill this need, a program was initiated at the University of Michigan under the sponsorship of the World Health Organization to synthesize promising drug candidates with potent antifilarial activity. After primary and secondary drug screening, methyl-(5-methoxybenzyl-1*H*-benzimidazol-2-yl) carbamate (UMF-058, I), a new benzimidazole carbamate and an analogue of MBZ, with potent antifilarial activity was recently synthesized [4]. It is a odourless white powder, which melts at 230–235°C with decomposition. It is very soluble in dimethyl sulphoxide, practically insoluble in acidic, basic and neutral buffers, and has a pK_a of 6.82. Following oral administration it is believed that is metabolized to MBZ.

In order to elucidate the pharmacokinetic properties of I, it was necessary to develop an assay for I and MBZ (Fig. 1) in biological fluids. A number of published methods exist for the quantification of MBZ. Alton *et al.* [5] developed a high-performance liquid chromatographic (HPLC) method to quantitate MBZ in plasma but this required multiple extraction steps and a large sample volume (2 ml). Another procedure,

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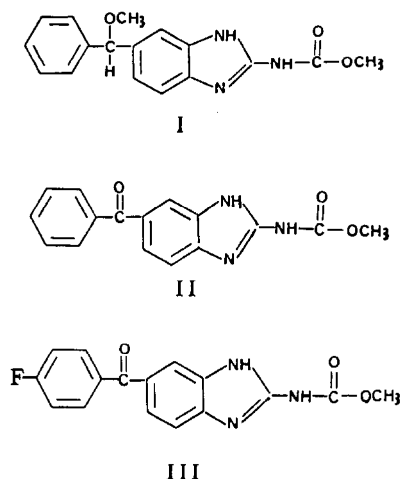

Fig. 1. Structures of UMF-058 (I), mebendazole (II) and flubendazole (III).

described by Allan *et al.* [6], possessed acceptable limits of detection but required a large sample volume (5 ml). In addition, this method also involved solid-phase extraction and hence increased expense. An HPLC method in combination with electrochemical detection has been reported [7], but this approach requires complex chromatographic conditions for the proper functioning of the detector. No HPLC method to quantitate I and MBZ in whole blood has been reported. This paper describes a cheap, rapid, sensitive and selective HPLC procedure for simultaneous determination of I and MBZ in whole blood samples.

EXPERIMENTAL

Chemicals

UMF-058 (I), MBZ [methyl-(5-benzoyl-1*H*-benzimidazol-2-yl) carbamate] and flubendazole (FBZ) [methyl-(5-(4-fluorobenzoyl)-1*H*-benzimidazol-2-yl) carbamate] were obtained from Dr. L. B. Townsend (University of Michigan, Ann Arbor, MI, USA). All chemicals and solvents were of analytical grade. HPLC-grade methanol and ammonium dihydrogenphosphate were purchased from Merck (Darmstadt, Germany). Diethyl ether, dimethyl sulphoxide (DMSO) and sodium chloride were purchased from May and Baker (Dagenham, UK), BDH (Poole, UK) and Riedel-de Haen (Hannover, Germany), respectively. Orthophosphoric acid was purchased from Ajax Chemicals (Auburn, Australia).

Chromatography

The analytical instrument used was a quaternary pump system (HP 1050, Walbronn, Germany), equipped with a syringe-loading sample injector with a 20-μl sample loop (Model 7125, Rheodyne, Cotati, CA, USA) coupled to a variable-wavelength UV detector (Lambda-Max Model 481, Waters Assoc., Milford, MA, USA) operated at 291 nm. The chromatograms were recorded using an electronic integrator (Model 3392A, Hewlett Packard, Avondale, PA, USA). Chromatographic separations were performed on a C_{18} reversed-phase stainless-steel column (150 mm × 3.9 mm I.D., 5 μm particle size; Nova Pak, Waters Assoc.) maintained at room temperature. The mobile phase was methanol–0.05 *M* ammonium dihydrogenphosphate (50:50, v/v) adjusted to pH 4.0 with orthophosphoric acid (17.2 *M*) at a flow-rate of 1 ml/min.

Extraction procedure

Extraction was carried out in 10-ml glass culture tubes pretreated with dichlorodimethylsilane in toluene (5%, v/v) in order to minimize adsorption on active sites on the glass. A 2-ml volume of saline solution (0.15 *M*) was added to a sample of whole blood (1.0 ml) containing the internal standard FBZ (100 ng, 10 μl), I and MBZ of various concentrations. The spiked blood sample was then vortex-mixed for 15 s. The mixture was extracted with diethyl ether (6 ml) by vortex-mixing for 1.5 min, and then centrifuged at 1000 *g* for 10 min. The supernatant (organic phase) was evaporated to dryness under a gentle stream of nitrogen at room temperature. The residue was reconstituted in DMSO (100 μl), and 20 μl were injected into the column.

Calibration

Stock solutions of I, MBZ (2.5–10 ng/μl) and the internal standard (10 ng/μl) prepared by dissolving each substance in DMSO were stored at

4°C. Calibration curves were prepared by spiking drug-free whole blood samples with standard solutions (25–500 ng, 10–50 μl) of I and MBZ and the internal standard (100 ng, 10 μl) to give a concentration range of 25–500 ng/ml. The samples were taken through the extraction procedure, and the peak-height ratio of the drug and its metabolite was plotted against the corresponding concentration of drug or metabolite. Linear regression of the peak-height ratio *versus* the drug or metabolite concentration was performed in order to estimate the slope, intercept (peak-height ratio for zero concentration) and correlation coefficient for each standard curve.

Analytical recovery, within-day and day-to-day precision

The absolute recoveries of the extraction procedure for I, MBZ and FBZ were determined by comparing the peak heights obtained from extracted samples (whole blood) containing known amounts of the substance with those obtained from equivalent amounts of the compounds in DMSO by direct injection. The within-day and day-to-day precision were determined by replicate assays of samples from spiked whole blood. The day-to-day assay variation was assessed over a period of five days.

Animal study

Leaf monkeys (*Presbytis cristata*) infected with *Brugia malayi* received 250 mg/kg I orally. Venous blood samples were taken before drug administration and thereafter at 0.5, 1, 2, 4, 6, 12, 24, 36, 48, 72, 96, 120 and 144 h. Blood was stored at −90°C until analysis.

Pharmacokinetic analysis

Data in the text are presented as mean ± S.D. values. The elimination half-life was calculated by regression analysis of the log linear portion of the whole blood concentration *versus* time curve. The area under the whole blood concentration–time curve (AUC) was calculated by the linear trapezoidal rule. Other pharmacokinetic parameters (clearance and volume of distribution) were calculated using Model-independent formulae [8]. Maximum concentration (C_{max}) and time to reach C_{max} (T_{max}) are the observed values.

RESULTS AND DISCUSSION

The extraction procedure, which was rapid and simple, permitted the compounds of interest to be isolated from whole blood without interference from endogenous substances under the given chromatographic conditions. The wavelength selected for the analysis, 291 nm, was the most suitable for simultaneous detection of I and MBZ because I exhibits maximum absorbance and MBZ exhibits a satisfactory absorbance at this wavelength, without interference from endogenous substances.

Fig. 2 illustrates the chromatograms of (a) a standard mixture, (b) drug-free monkey whole blood and (c) whole blood of a leaf monkey (*Presbytis cristata*) following the oral administration of I (250 mg/kg). The method yields clean chromatograms, with baseline resolution of MBZ, internal standard and I, at the retention times of 6.8, 8.5 and 10.1 min, respectively.

Mean recoveries from human whole blood were 92.0 ± 7.7% (range 84.8–97.6%) for I, 84.4 ± 4.4% (range 82.6%–86.4%) for MBZ and 87.5 ± 4.9% (range 86.0–89.1%) for FBZ (Table I).

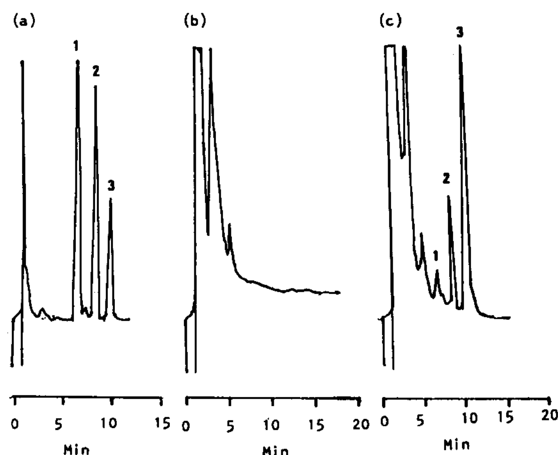

Fig. 2. Chromatograms of (a) a standard mixture of I, MBZ and FBZ, (b) drug-free leaf monkey whole blood (attenuation 0.05 a.u.f.s.) and (c) whole blood of a leaf monkey 36 h after oral administration of I (250 mg/kg) (attenuation 0.05 a.u.f.s.). Peaks: 1 = MBZ (4.7 ng); 2 = internal standard; 3 = I (261.4 ng).

TABLE I

MEAN RECOVERIES OF I, MBZ AND FBZ (n = 5)

Compound	Spiked blood concentration (ng/ml)	Recovery (%)	C.V. (%)
I	25	97.6	7.6
	100	93.4	2.8
	250	84.8	7.1
MBZ	25	82.6	7.3
	100	86.4	3.2
	250	84.3	4.6
FBZ	25	89.1	8.5
	100	87.5	3.6
	250	86.0	3.9

TABLE II

WITHIN-DAY PRECISION FOR ASSAY OF I AND MBZ (n = 5)

Compound	Spiked blood concentration (ng/ml)	Mean concentration determined (ng/ml)	C.V. (%)
I	50	50.2	2.9
	100	97.0	5.7
	250	254.5	4.1
MBZ	50	47.3	1.9
	100	94.9	2.7
	250	262.9	2.8

Using 1 ml of whole blood, the minimum detectable concentrations of I and MBZ were 7 and 6 ng/ml, respectively. The within-day coefficients of variation (C.V.) for I and MBZ were 2–6 and 1–3%, respectively (Table II), and the day-to-day C.V. were 5–6 and 4–6%, respectively (Table III). Calibration curves for both I and MBZ were linear in the range 0–500 ng/ml ($r > 0.998$). The equations of the calibration plots ($n = 5$) for I and MBZ were $y = 0.006x + 0.014$ and $y = 0.014x - 0.022$, respectively.

The validated method for the analysis of I and MBZ whole blood was used to study the pharmacokinetics of I and MBZ in a leaf monkey (*Presbytis cristata*) infected with *Brugia malayi* after a single oral dose (250 mg/kg) of the drug. The blood concentration–time profile over the period 0–144 h is shown in Fig. 3. The peak blood concentration of I (1898.7 ng/ml) was reached 24 h post-dose, and the AUC was 77 704.7 ng · h/ml. The clearance, volume of distribution and elimination half-life were 53.6 ml/min/kg, 119.4 l/kg and 25.7 h, respectively. MBZ could be detected in blood 4 h post-dose and reached a maximum concentration (63.9 ng/ml) at 12 h post-doze, with an AUC of 1367.4 ng · h/ml.

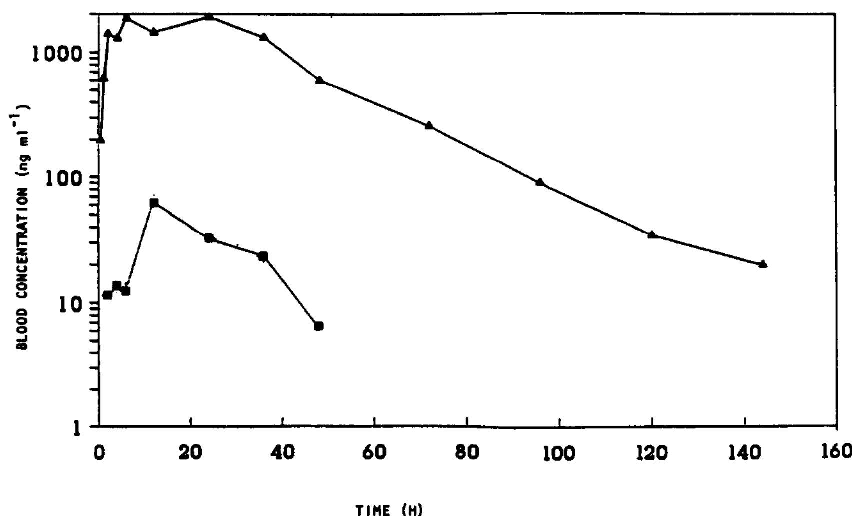

Fig. 3. Blood concentrations of I (▲) and MBZ (■) measured in leaf monkey (*Presbytis cristata*) infected with *Brugia malayi* following oral administration of a single dose (250 mg/kg) of I.

TABLE III

DAY-TO-DAY PRECISION FOR ASSAY OF I AND MBZ (n = 5)

Compound	Spiked blood concentration (ng/ml)	Mean concentration determined (ng/ml)	C.V. (%)
I	50	49.2	6.0
	100	101.0	5.3
	250	251.2	5.2
MBZ	50	47.7	5.3
	100	98.5	4.3
	250	247.2	5.2

The pharmacokinetic results show that the method has been applied successfully for the simultaneous assay of I and MBZ in whole blood. A number of published methods to quantitate benzimidazole carbamates in plasma have been reported [5,6,9,10]. However, the applicability of these methods to assay these compounds in whole blood is still unclear. The method described in this paper used whole blood to assay the compound of interest. In addition, this procedure worked equally well in plasma. This highlights an additional advantage of this method compared with other reported methods.

Pharmacokinetic studies in animal models require serial blood sampling. Since the total blood volume in animals is limited, the minimum amount of blood should be collected to ensure the survival of the animals during the study period. The method described here suits this purpose because only 1.0 ml of whole blood is needed for quantification of the compound of interest. In contrast, a larger volume of blood is needed to prepare an equivalent volume of plasma (1.0 ml) and therefore it is not suitable for pharmacokinetic studies. This highlights the limitations of other published methods [5,6,10] for application in such studies because these methods require large plasma samples (2.0–5.0 ml) to quantitate the compound of interest.

The extraction procedure developed is simple because it does not require pH adjustment prior to extraction. The short analysis time (10 min), together with rapid evaporation of diethyl ether at room temperature (28°C), allows rapid analysis, thus the method is suitable for routine analysis of a large number of samples. However, it is important to note that this method cannot detect other possible metabolites of I. However, it can be adapted to determine FBZ in whole blood using MBZ or I as an internal standard.

CONCLUSION

We have described an analytical method for the determination of I and MBZ that satisfies all the criteria required for an assay to be suitable for pharmacokinetic studies. It has several advantages, notably speed and low cost, compared with other published methods.

ACKNOWLEDGEMENTS

The study received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease (TDR). S.R. is in receipt of a Postgraduate Studentship from Universiti Sains Malaysia. We are grateful to Dr. L. B. Townsend for providing UMF-058, mebendazole and flubendazole standards, and to Marina Cheah for typing the manuscript.

REFERENCES

1 M. Dawson, P. A. Brathwaite, M. S. Roberts and T. R. Watson, *Br. J. Clin. Pharmacol.*, 19 (1985) 79.
2 R. J. Horton, *Parasitol. Today*, 6 (1990) 106.
3 J. E. F. Reynolds (Editor), *Martindale, The Extra Pharmacopoeia*, The Pharmaceutical Press, London, 29th ed., 1989.
4 S. Ram, D. S. Wise, L. L. Wotring, J. W. McCall and L. B. Townsend, *J. Med. Chem.*, 35 (1992) 539.
5 K. B. Alton, J. E. Patrick and J. L. McGuire, *J. Pharm. Sci.*, 68 (1979) 880.
6 R. J. Allan, H. T. Goodman and T. R. Watson, *J. Chromatogr.*, 183 (1980) 311.
7 B. Oosterhuis, J. C. F. M. Westeyn and C. J. Van Boxtel, *Ther. Drug Monit.*, 6 (1984) 215.
8 M. Rowland and T. N. Tozer, *Clinical Pharmacokinetics, Concepts and Applications*, Lea and Febiger, London, 1989.
9 M. T. Watts and V. A. Raisys, *J. Chromatogr.*, 230 (1982) 79.
10 G. Karlaganis, G. J. Munst and J. Bircher, *J. High Resol. Chromatogr. Chromatogr. Commun.*, 2 (1979) 141.