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Short Communication

A rapid high-performance liquid chromatographic procedure for the determination of triclabendazole and its metabolites in sheep plasma

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Introduction

Triclabendazole (4-chloro-5-(2,3-dichlorophenoxy)-2-methyl-thiobenzimidazole, coded CGA-89317) is a benzimidazole anthelmintic with specific activity against liver fluke infections in sheep and cattle [1, 2]. Preliminary studies on whole blood from sheep treated with triclabendazole revealed the virtual absence of the parent compound and the presence of two additional compounds. These subsequently were identified as the sulphoxide (CGA-110752) and sulphone (CGA-110753) metabolites of triclabendazole (Ramsteiner, CIBA-GEIGY Switzerland, unpublished communication). Their structures are shown in Fig. 1. In addition it has been found that triclabendazole and its metabolites were associated with the plasma rather than the red cells of the blood (unpublished data). Therefore methods developed to analyse the drug and its metabolites in the bloodstream need only be applied to plasma.

Since detailed pharmacokinetic studies of the drug in ruminants required a large number of blood samples to be analysed, a simple and cost effective procedure was sought. In common with other investigations on the pharmacokinetics of benzimidazole anthelmintics [3, 4], high-performance liquid chromatography (HPLC) was selected as the means of quantifying the compounds in plasma extracts. The present paper describes the procedures adopted in the analytical method and presents data obtained by application of the method to the analysis of sheep plasma.

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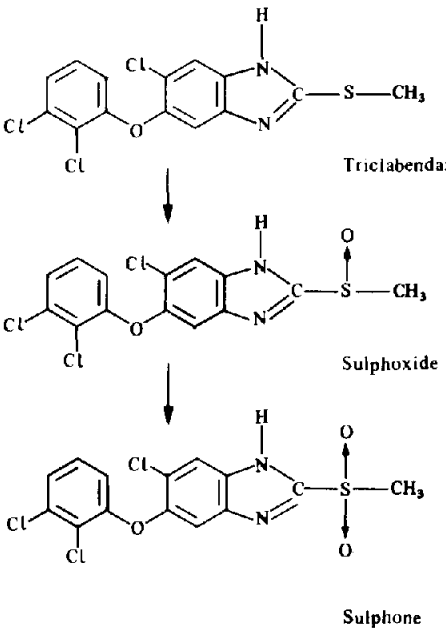

Figure 1
Chemical structure of triclabendazole and its sulphoxide and sulphone metabolites.

Material and Methods

Chemicals

Analytical standards of triclabendazole, its sulphoxide and sulphone metabolites were obtained from CIBA-GEIGY Limited (Agricultural Division, Basel, Switzerland). Reagents were of analytical grade; acetone, methanol and water were redistilled in glass before use. Solvents used for HPLC were filtered through a 0.45-μm Sartorius membrane filter.

Reagents

Two reagents necessary either in the HPLC mobile phase or to render the sample extracts suitable for HPLC analysis were prepared as follows:

Reagent 1: Glacial acetic acid (120 ml) was added to ammonium acetate (200 g) and diluted to 1 l with water;

Reagent 2: Reagent 1 (9 ml) was diluted to 100 ml with water.

Extraction and clean-up procedure

Plasma (5 ml) was transferred by pipette into a 10-ml polypropylene centrifuge tube and acetone (5 ml) was added by pipette down the inside wall of the tube. The tube was capped, inverted immediately, shaken vigorously until the protein had precipitated evenly (about 30 s), and centrifuged (1500 **g** for 15 min). An aliquot (5 ml) of the supernatant was transferred to another centrifuge tube containing water (5 ml). The contents were mixed, transferred to a glass syringe (10 ml) and passed through a C_{18} Sep Pak (Waters Associates Part 51910) which had been conditioned previously with methanol (10 ml) and water (10 ml). A further 10 ml of water was used to rinse the centrifuge tube and syringe; these rinsings were also passed through the Sep Pak. Methanol (3 ml) by pipette was used to rinse the syringe and elute the compounds from

the Sep Pak into a tube containing reagent 2 (1 ml). The contents were mixed and filtered through a 0.45-μm membrane filter into a vial before analysis by HPLC.

Liquid chromatography

The reversed-phase HPLC system (Waters Associates Inc. Milford, USA) comprised a 6000A solvent delivery pump, a 710B Wisp sample processor, a 300 × 3.9 mm i.d. column packed with 10-μm C_{18}-microBondapak, and a Lambda-Max Model 481 LC spectrophotometer detector set at 300 nm. A Hewlett–Packard 3390A integrator was used to quantify the peaks. The mobile phase was methanol–water–reagent 1–chloroform (750:200:25:10, v/v/v/v) and the flow-rate was 1 ml/min. The retention times were 9 min for triclabendazole, 7 min for its sulphoxide and 6 min for the sulphone. Concentrated solutions (1000 μg/ml) of each compound were prepared in methanol; from these solutions a range of three-component standards at concentrations between 0.1 and 10 μg/ml was prepared in the mobile phase. The concentration of the compound in the cleaned-up plasma extracts (40 μl injections) was determined by comparing the detector response for each compound in the sample with that of the corresponding peak in the standard mixture.

Preliminary kinetic study

To evaluate the suitability of the method to detect triclabendazole and its metabolites during pharmacokinetic studies, triclabendazole (as the commercial formulation Fasinex 50, CIBA-GEIGY) was administered orally to five sheep at a dose of 10 mg/kg. Blood (20 ml) was withdrawn from the jugular vein, at appropriate intervals to obtain a concentration profile, and transferred to tubes containing EDTA as an anticoagulant. Plasma was separated by centrifuging at a 1500 **g** for 15 min and immediately stored at −18°C until analysed.

Results and Discussion

When control samples of sheep plasma were analysed by the HPLC method no significant peaks interfering with any of the three compounds were found (Fig. 2). The limit of determination for each of the compounds was set at 0.10 mg/l. The percentage recovery of each compound from control plasma to which a multi-component standard in dimethyl sulphoxide had been added is summarised in Table 1. A representative chromatogram of plasma spiked with 0.2 μg/ml of each analyte is shown in Fig. 2 to demonstrate the quality of resolution and peak symmetry.

The average recovery ($n = 6$) for triclabendazole over the range 0.1–1.0 μg/l was 102%; recoveries ($n = 6$) for the sulphoxide and sulphone metabolites over the range 0.1–10 μg/l were 108% and 110%, respectively.

Figure 3 shows the plasma profiles for the sulphoxide and sulphone metabolites of triclabendazole from plasma samples of sheep treated with triclabendazole (as Fasinex) at 10 mg/kg. The maximum plasma level for the sulphoxide was attained 16 h after the treatment was given whereas the peak sulphone value was reached 8 h later. At no time after treatment could unchanged triclabendazole be found in the plasma. The absolute levels of the metabolites of triclabendazole are much higher than those that have been found with corresponding metabolites of fenbendazole or albendazole [5, 6]. This difference between triclabendazole and other benzimidazole anthelmintics will be the subject of a separate article.

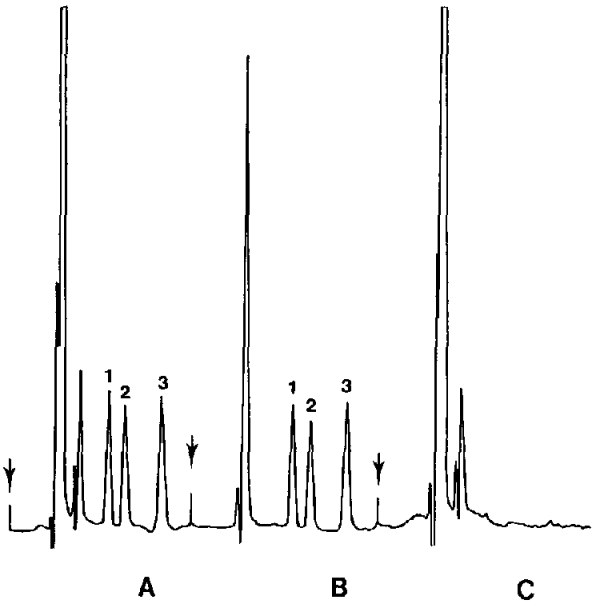

Figure 2
HPLC chromatogram of triclabendazole and its metabolites in sheep plasma. (A) Sheep plasma extract (25 mg injected) to which had been added 1 μg/ml each of 1 = triclabendazole sulphone, 2 = triclabendazole sulphoxide, 3 = triclabendazole (0.2 μg/ml recovery); (B) Standard solution in mobile phase (5 ng of each); 1 = triclabendazole sulphone, 2 = triclabendazole sulphoxide, 3 = triclabendazole; (C) Untreated sheep plasma extract (25 mg injected).

Table 1
Recovery and precision for the determination of triclabendazole and its metabolites in sheep plasma

Added concentration (μg/ml)	% Recovery ± RSD*		
	CGA-89317	CGA-110752	CGA-110753
0.1	98 ± 2	109 ± 7	116 ± 4
0.2	105 ± 5	106 ± 2	107 ± 3
1.0	104 ± 2	106 ± 2	107 ± 4
10.0	—	110 ± 1	110 ± 3

*RSD = Relative standard deviation (n = 6).

The extraction and clean-up procedures described in the present paper require minimum bench space, negligible glassware and solvent consumption. Furthermore the C_{18} Sep Pak can be re-cycled at least 12 times without loss of performance. Up to 25 samples can be processed by one technician per day; in contrast, the restrictions of the conventional extraction and solvent partitioning procedures permit analysis of only 12 samples per day.

Although triclabendazole and its metabolites can be successfully quantified on a silica absorption HPLC column [7] it has been found more convenient to couple the C_{18} Sep Pak clean-up steps to reversed-phase chromatography, thereby avoiding the evaporation

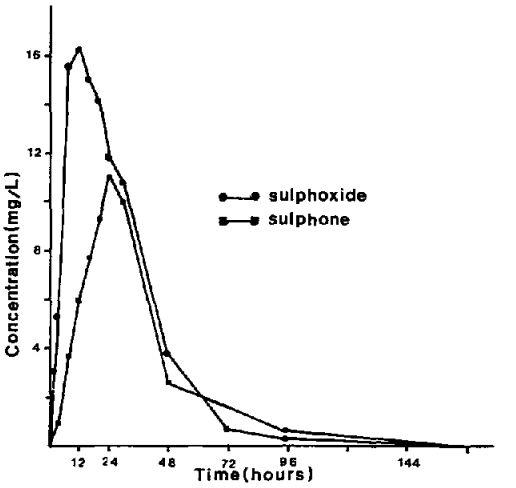

Figure 3
Plasma levels of triclabendazole sulphoxide and sulphone metabolites in sheep after oral administration of triclabendazole at 10 mg/kg.

needed in reconstituting the residue into a normal phase solvent. Alvinerie and Galtier in their work with albendazole [4] and triclabendazole [7] did not clean up their crude extract prior to evaluation by HPLC. In the present study premature loss of separation efficiency was experienced with the reversed-phase column when crude preparations containing the metabolites of triclabendazole were injected on to the HPLC column. Therefore the inclusion of a clean-up step in the analysis of sheep plasma for the metabolites of triclabendazole is considered to be obligatory.

The analytical procedure described in the present paper is intended as a rapid screening method for the metabolites of triclabendazole in the plasma of ruminants. Tests have shown that the method works equally well for bovine plasma as it does for sheep plasma. The precision shown in Table 1 was considered quite acceptable for the uses to which the method may be applied. If greater precision is required then greater control of volumes eluted from the Sep Pak would need to be enforced.

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