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## Note

# Assay of triclabendazole and its main metabolites in plasma by high-performance liquid chromatography

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Triclabendazole [5-chloro-6-(2,3-dichlorophenoxy)-2-methylthiobenzimidazole] is a potent member of the benzimidazole group. This drug has been tested in sheep, goats and cattle and has been found to be effective against both larval and adult strips of *Fasciola hepatica* [1]. More recently [2], the detrimental effect of triclabendazole against *Fasciola magna* in naturally infected calves was demonstrated.

Bovine and ovine fascioliasis are an important cause of liver disease in abattoirs and lowered productivity in herds in many areas of the world [2]. Control of the infestion may be achieved by use of anthelmintic treatment of the infected animals. The purpose of the present study was to develop a rapid and simple high-performance liquid chromatographic (HPLC) method for assaying triclabendazole and its metabolites in plasma during pharmacokinetic studies.

## EXPERIMENTAL

### *Chemicals and reagents*

All reagents were analytical-grade purity; ethanol and acetic acid were purchased from Merck (Darmstadt, F.R.G.), hexane and ethyl acetate originated from Farmitalia Carlo Erba (Milan, Italy), water was deionized and distilled.

Triclabendazole and its metabolites (sulphoxide and sulphone) were kindly provided by Dr. C. Vallas (Arkovet, Ciba Geigy, Paris, France). Oxfendazole

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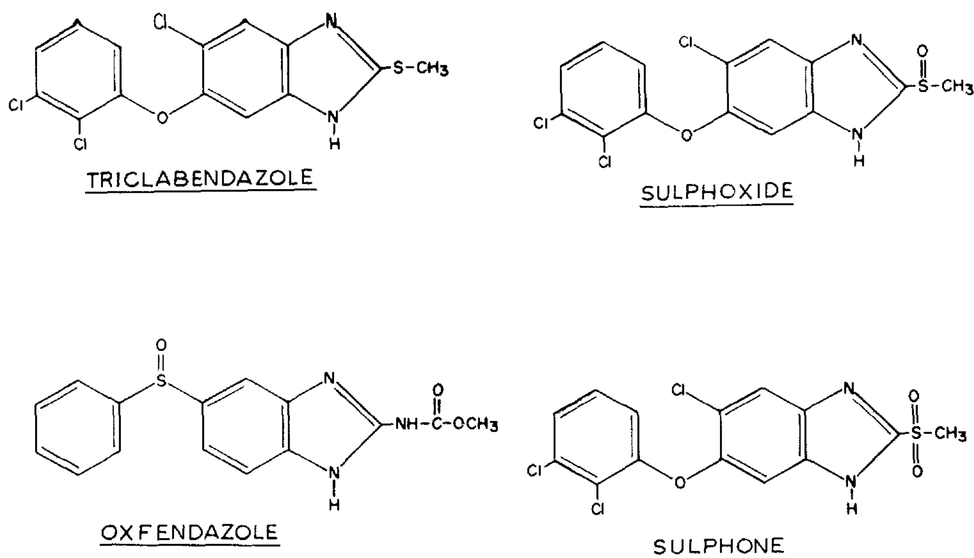


Fig. 1. Chemical structures of the benzimidazoles investigated.

was kindly provided by Professor Delatour (École Nationale Vétérinaire, Lyon, France). The structures of these compounds are shown in Fig. 1.

*Apparatus*

A constant-flow high-performance liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.) consisting of a Model M6000 pump, a WISP 710B automatic injector and a Model M 481 spectrophotometer was connected to an LCI 100 laboratory computing integrator (Perkin Elmer, Norwalk, CT, U.S.A.). The column (250 $\times$ 4.6 mm I.D.) was packed with Partisil silica gel (5 $\mu$m) (Whatman SA, Paris, France).

*Standards*

The solutions of triclabendazole, sulphoxide and sulphone used to construct the standard curves were prepared by serial dilution of a stock solution containing 1.0 mg/ml ethanol. A stock solution of the internal standard (oxfendazole) was prepared in ethanol (1 mg/ml). A working standard was prepared by diluting 1.0 ml of this solution to 100.0 ml with ethanol to give a solution containing 10 $\mu$g/ml.

*Operating conditions*

The mobile phase was hexane—ethanol—glacial acetic acid (500:50:0.6). This solution was passed through a 0.45-$\mu$m filter (Whatman, Maidstone, U.K.) and degassed with a gentle stream of helium during analysis.

The flow-rate was maintained at 1.5 ml/min, giving a back-pressure of 75 bar at room temperature. The UV detector was set at 215 nm.

*Extraction procedure*

Plasma (100 $\mu$l), 10 $\mu$l of the working solution of the internal standard (equivalent to 0.1 $\mu$g of oxfendazole) and ethyl acetate (1 ml) were added

to a 1.5-ml polypropylene tube. The samples were shaken on a table-top shaker for 15 min and centrifuged for 2 min at 5000 *g*. The organic layer was aspirated, transferred to a 15-ml centrifuge tube and evaporated to dryness under a stream of nitrogen gas to prevent oxidation. The sample extract was reconstituted with 100 μl of the eluent and an 80-μl aliquot was injected on the HPLC column.

*Calibration*

Standard calibration curves for triclabendazole, sulphoxide and sulphone over the range 0.1–4 μg/ml were prepared using drug-free serum and 0.1 μg of oxfendazole as internal standard. Pooled serum samples were run through the procedure and calibration curves were constructed using the ratio of analyte to internal standard peak area, calculated as a function of analyte concentration. Least-squares regression analysis was used to determine the slope, intercept and correlation coefficient for each compound in the concentration range tested (Table II). The response of the HPLC system was linear from 0.1 to 4 μg/ml for each of the three compounds.

TABLE I

RETENTION TIMES AND CAPACITY FACTORS OF THE BENZIMIDAZOLES STUDIED

| Compound | Capacity factor | Retention time (min) |
|---|---|---|
| Triclabendazole | 1.85 | 5.69 |
| Triclabendazole sulphone | 2.4 | 6.80 |
| Triclabendazole sulphoxide | 3.96 | 9.92 |
| Oxfendazole | 5.52 | 13.04 |

TABLE II

LEAST-SQUARES REGRESSION STATISTICS FOR HPLC CALIBRATION DATA OF TRICLABENDAZOLE AND ITS SULPHOXIDE AND SULPHONE METABOLITES IN SPIKED SAMPLES OF SHEEP PLASMA, USING OXFENDAZOLE AS INTERNAL STANDARD

| Component | Slope (mean ± S.D.) | Intercept (mean ± S.D.) | Correlation coefficient ($n$ = 5) |
|---|---|---|---|
| Triclabendazole | 0.987 ± 0.042 | 0.0037 ± 0.041 | 0.9998 |
| Triclabendazole sulphone | 1.814 ± 0.089 | 0.015 ± 0.0053 | 0.9997 |
| Triclabendazole sulphoxide | 1.756 ± 0.045 | 0.0050 ± 0.0085 | 0.9998 |

Extraction efficiency of the benzimidazoles was measured by comparing the peak area from the analysis of serum samples that were spiked with 1 μg of triclabendazole, sulphoxide and sulphone, to the peak area resulting from a direct injection.

*Precision*

The precision of the extraction procedure and chromatography was evaluated by processing as replicates, on different days, aliquots of pooled

TABLE III

INTER-ASSAY PRECISION OF THE HPLC METHOD FOR THE DETERMINATION OF TRICLABENDAZOLE AND ITS METABOLITES

| Component | Theoretical concentration (μg/ml) | Concentration found (mean ± S.D., $n$ = 5) (μg/ml) | Coefficient of variation (%) |
|---|---|---|---|
| Triclabendazole | 1.0 | 1.040 ± 0.063 | 6.3 |
| Triclabendazole sulphone | 1.0 | 0.987 ± 0.032 | 3.2 |
| Triclabendazole sulphoxide | 1.0 | 0.984 ± 0.046 | 4.6 |

sheep serum samples containing known amounts of triclabendazole, sulphoxide and sulphone (Table III).

*Drug disposition study*

In order to test the ability of this method to measure triclabendazole and its metabolites in the course of pharmacokinetic studies, triclabendazole was administered orally to a sheep at a dose-rate of 10 mg/kg. Blood was withdrawn via the jugular vein at 1, 2, 4, 6, 8, 12, 24 h and every day for two weeks after injection and transferred to tubes containing 10 U of heparin. The plasma was separated immediately and stored at —20°C until analysis.

## RESULTS AND DISCUSSION

*Chromatography*

Using the described chromatographic conditions, triclabendazole sulphone, sulphoxide and the internal standard oxfendazole were well resolved with no interference from endogenous compounds; typical chromatograms are shown in Fig. 2.

Normal-phase liquid chromatography showed a high selectivity in the separation of benzimidazole; a good separation was obtained in 15 min (Table I).

Extraction recoveries were 89.5, 82.2 and 83.1, respectively, with relative standard deviations of 3.1, 4.9 and 3.5 ($n$ = 5).

Finally, the analytical procedure based on microparticulate silica permits the separation of triclabendazole and its metabolites: moreover, other benzimidazoles such as albendazole have been successfully separated from the metabolites by means of a similar chromatographic system [3]. Another assay [4], using reversed-phase chromatography, can also be used for the determination of oxfendazole.

The detection limit of the proposed method was 50 ng/ml for all products, based on 100-μl plasma samples; this could be enhanced by increasing the amount of sample.

In conclusion, a selective and sensitive method has been developed for monitoring triclabendazole and its metabolites during pharmacokinetic studies.

*Kinetic studies*

Fig. 3 presents a typical plasma concentration—time profile of triclabendazole sulphoxide and sulphone after oral administration of tri-

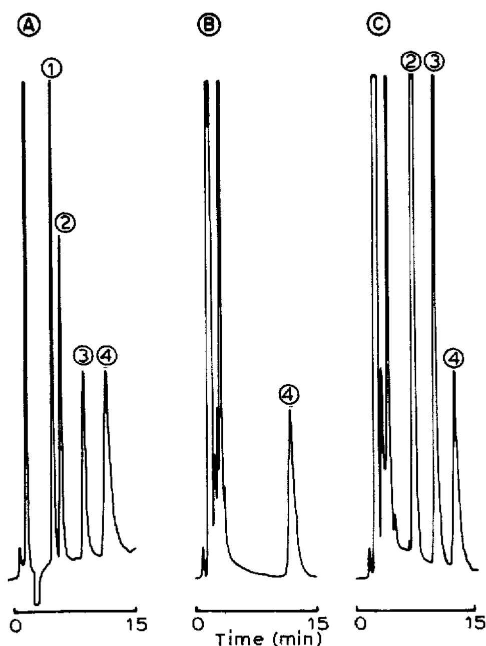


Fig. 2. (A) Chromatogram of a mixture of 100 ng each of triclabendazole, sulphone, sulphoxide and oxfendazole; (B) chromatogram of a blank sheep plasma spiked with internal standard at 1.0 μg/ml; (C) chromatogram of a plasma sample from an animal 36 h after treatment with triclabendazole. Peaks: 1 = triclabendazole; 2 = sulphone; 3 = sulphoxide; 4 = oxfendazole.

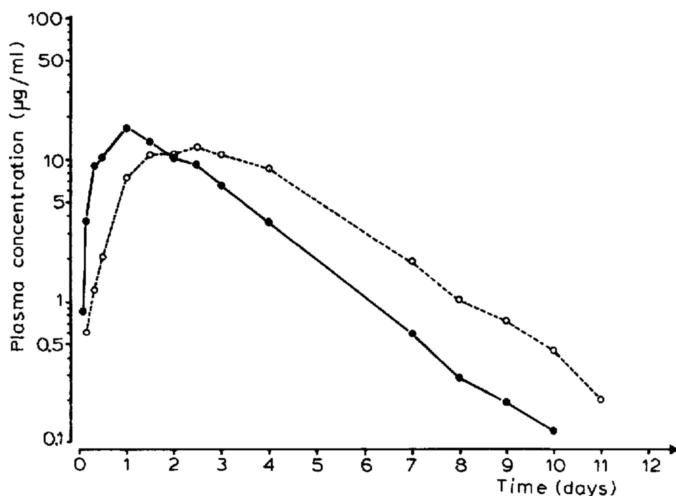


Fig. 3. Plasma concentration—time curve in a sheep given a 10 mg/kg oral dose of triclabendazole. (•) Triclabendazole sulphoxide; (○) triclabendazole sulphone.

clabendazole to a sheep. As previously described [5] in the pharmacokinetic study of the related drug albendazole orally administered in sheep, the parent sulphur drug triclabendazole did not appear in plasma at any time in concentrations higher than the detection unit. Moreover, the metabolite triclabendazole

sulphoxide, which could represent one of the major therapeutically active metabolites, peaked in plasma 24 h after administration with a concentration of 16 μg/ml. Triclabendazole sulphone, the terminal product of metabolism, appears later than sulphoxide and peaks 48 h after administration. The concentrations of these two metabolites decreased very slowly for twelve days. The elimination half-lives of the two metabolites calculated from the slope of the terminal linear portion of the serum concentration—time curve were ca. 30 h for the two products. The results presented demonstrate that the method is appropriate for pharmacokinetic studies.

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