Analysis of Benzimidazoles in Body Fluids by High-Performance Liquid Chromatography

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Abstract □ A simple assay for benzimidazole in plasma and GI fluids using high-performance liquid chromatography was developed. The benzimidazoles were extracted using ether and were chromatographed with methanol–ammonium carbonate mixtures as the eluting solvents. Benzimidazole was detectable in 4 ml of plasma or GI fluid to a limit of 20 ng/ml.

Keyphrases □ Benzimidazoles—analysis, high-performance liquid chromatography, GI fluids, plasma □ High-performance liquid chromatography—analysis, benzimidazoles, GI fluids, plasma □ Anthelmintics—benzimidazoles, high-performance liquid chromatographic analysis, GI fluids, plasma

The benzimidazole anthelmintics are characterized by low mammalian toxicity and a broad spectrum of activity against GI nematodes. Some members of the group also are active against lungworms, tapeworms, and liver flukes in humans, cattle, sheep, and other animals. This paper describes a simple assay for this important group of anthelmintics using high-performance liquid chromatography (HPLC).

BACKGROUND

The anthelmintic activity of the benzimidazole anthelmintics appears to be the result of the length of time over which the parasite is exposed to the drug rather than the peak concentration to which the parasite is exposed. Thus, administration of the drug in divided doses usually increases anthelmintic activity (1). The drugs exert their activity on the parasites by inhibiting glucose uptake (2) and/or by inhibiting the enzyme fumarate reductase (3). Differences between members of the group appear to be more a result of differing pharmacokinetics of the drugs in the host animal than of different inherent anthelmintic activity. However, there is a lack of data on the pharmacokinetics of these drugs, probably because of analytical problems.

Most published data on benzimidazole pharmacokinetics are from experiments using radiolabeled drug without distinguishing between the parent drug and the metabolites. A method was published for the analysis of fenbendazole using fluorescence spectrophotometry, but this method also fails to distinguish the parent drug from the metabolites (4). A radioimmunoassay for oxfendazole (5) was reported recently, but this complex method requires the raising of an antiserum, and some cross-reactivity with metabolites occurs.

This paper describes a sensitive HPLC method for the analysis of oxfendazole, fenbendazole, and albendazole, the most potent members of the group, in body fluids. The method can be adapted for the other five members of the group that are marketed in the United Kingdom.

EXPERIMENTAL

Materials—Analar reagents were used, and the HPLC solvents were distilled.

Extraction—The method is applicable to plasma, rumen fluid, abomasal fluid, and duodenal fluid.

To 4 ml of plasma or GI fluid in a 50-ml stoppered tube were added 4 ml of phosphate buffer (pH 7.4) and 20 ml of ether. The tube was shaken for 10 min on a rotary mixer. Then 16 ml of the ether layer was removed to a clean test tube, and 20 ml of ether was added to the first tube, which was shaken again for 10 min. Twenty milliliters of ether was removed and combined with the initial 16 ml of ether.

The ether was evaporated slowly on a dry bath at 60° under nitrogen to a volume of about 6 ml and then was transferred to a conical 10-ml glass tube. The 50-ml tube was washed with several milliliters of ether, and the washings were added to the conical tube. The ether then was evaporated to dryness, the walls of the conical tube were rinsed with ether, and the solution was evaporated again to dryness. The residue in the tube was redissolved in 50 μl of methanol with the aid of an ultrasonic bath. If higher concentrations were expected, larger volumes of methanol were used. Five microliters of this solution was used for HPLC analysis.

HPLC—The HPLC system consisted of a solvent delivery pump[1] and a variable-wavelength UV detector[2] with a 100 × 8-mm column[3] packed

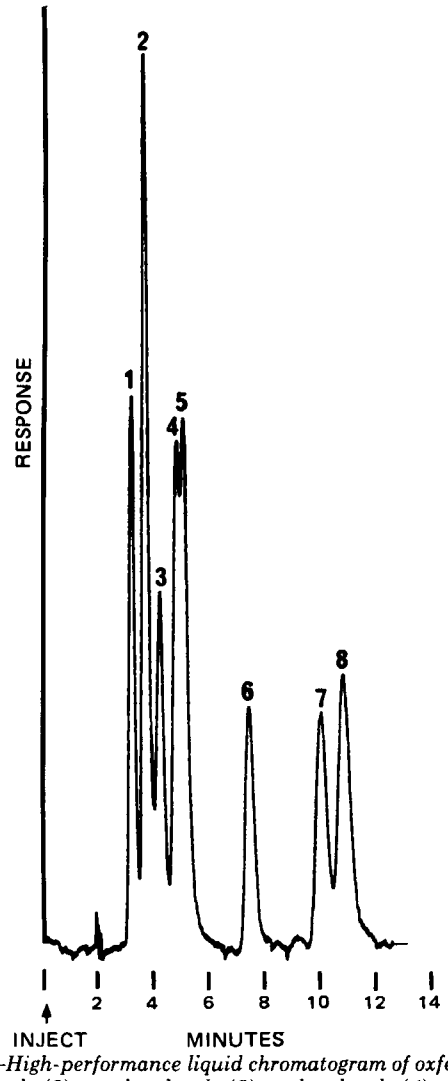

Figure 1—*High-performance liquid chromatogram of oxfendazole (1), thiabendazole (2), cambendazole (3), mebendazole (4), oxibendazole (5), albendazole (6), fenbendazole (7), and parbendazole (8) in methanol–ammonium carbonate (0.05 M) (65:35). Five microliters of a solution containing 10 μg/ml of each benzimidazole in methanol was used for injection.*

[1] Model 110, Altex, Berkeley, Calif.
[2] Model CE 2012, Cecil Instruments, Cambridge, England.
[3] Shandon Southern Products Ltd., Runcorn, Cheshire, England.

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Table I—Recoveries of Benzimidazoles added to Plasma, Rumen Fluid, and Abomasal Fluid

Compound	Recovery, %		
	Plasma	Rumen Fluid	Abomasal Fluid
Fenbendazole	83–100 (n = 10)	82–105 (n = 9)	83–112 (n = 8)
Oxfendazole	70–97 (n = 10)	70–97 (n = 6)	72–92 (n = 12)
Albendazole	73–103 (n = 9)	72–90 (n = 4)	87–93 (n = 4)

Table II—Estimation of Oxfendazole in Abomasal Fluid

Oxfendazole Added, μg	Oxfendazole Measured, μg	Mean ± SE	SEM, %	Ratio of Assayed to Added
1.5	1.03	1.08 ± 0.03	2.8	0.72
	1.08			
	1.13			
7.5	5.90	6.07 ± 0.09	1.5	0.81
	6.20			
	6.10			
15	12.10	11.57 ± 0.27	2.3	0.77
	11.20			
	11.40			
30	24.50	24.93 ± 0.59	2.4	0.83
	24.20			
	26.10			

with ODS-Hypersil 10[4]. The solvent flow rate was 1.5 ml/min, and the detector wavelength was 292 nm.

The drug concentration in the samples was calculated with the aid of calibration curves prepared by adding known drug amounts (0.05–10 μg/ml) to blank plasma or GI fluids. The standard samples were run through the procedure, and the peak heights of drug in the sample were compared with the calibration curve prepared from the standard samples.

RESULTS AND DISCUSSION

The new broad-spectrum benzimidazoles have limited solubility in both water and organic solvents. While this feature enhances their action by slowing drug dissolution after oral dosage, it also produces difficulties in analysis because the concentrations found in plasma and in the GI contents are low and are present for long periods. The method described here is sensitive and practical for the evaluation of the pharmacokinetics of the benzimidazoles.

The retention times of the benzimidazoles varied considerably, and Fig. 1 shows a chromatogram of the eight benzimidazoles using a solvent mixture of methanol—ammonium carbonate (0.05 M) (65:35). For the analysis of individual benzimidazoles with longer retention times, retention times can be shortened by increasing the proportion of methanol in the solvent mixture. For example, parbendazole has a retention time of 3.6 min in 85:15 methanol–ammonium carbonate.

Recoveries were determined by measuring standard concentrations (0.2–5.0 μg/ml) of some benzimidazoles added to plasma, rumen fluid, and abomasal fluid (Table I). The method is sufficiently sensitive to measure plasma and GI fluid concentrations down to 0.02 μg/ml with 4 ml of sample or 0.01 μg/ml with 8 ml of sample.

The accuracy and precision of the method for oxfendazole were determined by adding known amounts of oxfendazole to 4 ml of abomasal fluid. Samples of each known concentration then were assayed in triplicate (Table II). It was determined that the recovery losses were due to the extraction procedure rather than to benzimidazole degradation.

Fenbendazole, oxfendazole, and albendazole, which are the most potent compounds of the benzimidazole series and have the broadest spectrum of activity, were administered at 10 mg/kg to three sheep with permanent ruminal and abomasal fistulae. Samples of plasma, ruminal fluid, and abomasal fluid were withdrawn at intervals for measurement of the benzimidazole concentration[5].

[4] Whatman Co., Clifton, N.J.

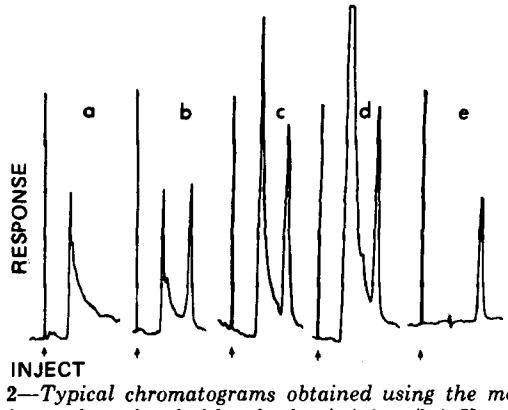

Figure 2—*Typical chromatograms obtained using the method on samples from a sheep dosed with oxfendazole (10 mg/kg). Key: a, plasma before oxfendazole administration; b, plasma at 12 hr after administration; c, abomasal fluid at 12 hr; d, ruminal fluid at 12 hr; and e, oxfendazole standard (10 μg/ml).*

Under the conditions described, no interfering substances were encountered in any fluid. The peaks encountered were symmetrical and, except at the lowest concentrations, were well defined. Typical chromatograms for oxfendazole are shown in Fig. 2.

The mean peak concentrations encountered in the three sheep for oxfendazole were 0.8, 0.4, and 4.6 μg/ml in plasma, ruminal fluid, and abomasal fluid, respectively. Oxfendazole was detectable for up to 11 days after administration. For fenbendazole, the mean peak concentrations were 0.13, 3.4, and 2.6 μg/ml, respectively, and it was detectable in all sheep 120 hr after administration. Fenbendazole was metabolized to oxfendazole (its equivalent sulfoxide metabolite), and the kinetics of the oxfendazole formed were similar to those of the administered oxfendazole.

Albendazole was not detectable in any plasma samples, although it could be detected in ruminal and abomasal fluids at maximum concentrations of 0.3 and 4.9 μg/ml, respectively. Albendazole was detectable in ruminal and abomasal fluids for at least 72 hr after administration. Metabolites (sulfoxide and sulfone) were found in high concentration in the plasma and abomasum after albendazole administration and were present for more than 48 hr. These metabolites did not interfere with the assay of the parent drug, but they also could be assayed by this method.

[5] A full description of the pharmacokinetics of these drugs in sheep will be the subject of a separate investigation.

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