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Highly Sensitive Ion Pair Liquid Chromatographic Determination of Albendazole Marker Residue in Animal Tissues

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A simple, rapid, and highly sensitive ion pair liquid chromatographic method for the determination of albendazole sulfoxide, albendazole 2-aminosulfone, and albendazole sulfone, which constitute the marker residue of albendazole in animal tissues (muscle, fat, liver, and kidney), is described. Tissue samples were extracted with acetonitrile, and the extracts were partitioned, as ion pairs, into dichloromethane. The organic layer was evaporated to dryness, and the residue was reconstituted in phosphate buffer and extracted with ethyl acetate. Separation was carried out isocratically with a mobile phase containing both positively and negatively charged pairing ions. Detection was performed fluorometrically, with excitation and emission wavelengths set at 290 and 320 nm, respectively. Overall recoveries were better than 76%, and the overall relative standard deviation was better than 7.3% in all tissues examined. The limits of quantification were 20, 1, and 0.5 ng/g for sulfoxide, 2-aminosulfone, and sulfone metabolites, respectively. The method was successfully applied to determine residues in tissues of two sheep orally administered an albendazole formulation.

KEYWORDS: Albendazole; residues; animal tissues; ion pair chromatography; fluorescence detection

INTRODUCTION

Albendazole is a benzimidazole carbamate, which has proved to be a very effective and efficient anthelmintic in animals. Following oral dosing to farm animals, albendazole is readily absorbed from the gut and rapidly metabolized by oxidation of its sulfide group to form albendazole sulfoxide and albendazole sulfone and by deacetylation of the carbamate group to form albendazole 2-aminosulfone (*1*). Because these metabolites, which constitute the marker residue of albendazole, may manifest themselves as residues in tissues from treated animals posing a health hazard to consumers (*2*, *3*), maximum residue limits (MRLs) of 100 μg/kg for muscle and fat, 500 μg/kg for kidney, and 1000 μg/kg for liver tissues have been set by the European Union (*4*).

A number of analytical methods have been developed to ensure the safety of marketed tissues. However, a literature survey shows that most analytical methods for measuring violative albendazole residues in animal tissues are limited to the determination of either the parent compound (*5*−*7*) or its 2-aminosulfone metabolite (*8*−*10*). A multiresidue method that is capable of individually measuring albendazole and its major metabolites in fish muscle has recently been reported (*11*), but possible matrix interference when analyzing tissues from other species cannot be excluded. A confirmatory liquid chromatographic mass spectrometric method for quantitative determination of benzimidazole residues in muscle tissue has also been described (*12*). This method, although valuable for confirmatory purposes, cannot be used on a routine basis to detect albendazole residues in unknown tissue samples due to the high cost of the analysis. Moreover, both methods (*11*, *12*) have been validated for use only in muscle tissue and not in other target tissues (fat, liver, and kidney), which have to be routinely analyzed (*4*) in residue monitoring or depletion studies. It is worth noting that liquid chromatography (LC) and tandem mass spectrometry might be an interesting alternative for the determination of albendazole residues in target tissues, taking into account the high specificity that it provides, which might help to considerably reduce sample preparation and working time.

An efficient methodology for the determination of albendazole marker residue in all target tissues does not currently exist; however, the ability to regulate the presence of this anthelmintic in animal tissues depends on such a methodology. In this regard, we have developed and validated a simple and cost effective liquid chromatographic method for the accurate and precise determination of albendazole sulfoxide, albendazole 2-aminosulfone, and albendazole sulfone in all target tissues, at levels well below the established MRLs. The method was successfully applied to determine residues in muscle, liver, kidney, and fat tissues of two sheep treated with albendazole.

MATERIALS AND METHODS

Apparatus. A modular liquid chromatographic system (Gilson Medical Electronics, Villiers-le-Bel, France) consisting of a model 305 piston pump, a model 805 manometer, and a model TC 831 column

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oven was used in this study. Injections were made through a 7125 Rheodyne valve (Rheodyne Inc., Cotati, CA) equipped with a 20 μL loop. A RF 551 fluorescence detector (Shimadzu Corp., Kyoto, Japan) with excitation and emission wavelengths set at 290 and 320 nm, respectively, was used to detect the signal response. The detector was linked to a BD 111 Kipp & Zonen (Delft, Holland) pen recorder. The stationary phase was Nucleosil 100-5 C_{18}, 5 μm material in a Mackerey-Nagel LC column (Germany), 250 mm × 4.6 mm id.

A Milli-Q purification system (Millipore, Bedford, MA) was used for ultrapurification of tap water, and an Ultra-Turrax (Janke & Kunkel, GmbH, Germany) high-speed blender, a G-560E vortex mixer (Scientific Industries, Inc., Bohemia, NY), a Centra-MP4 centrifuge (IEC, Needman Heights, MA), and a ReactiTherm evaporation unit (Pierce Chemicals, Rockford, IL) were used for sample treatment.

Reagents. The high-performance liquid chromatography grade acetonitrile and reagent grade ethyl acetate, isooctane, dimethyl sulfoxide, dichloromethane, *ortho*-phosphoric acid, disodium hydrogen phosphate, octanesulfonate sodium salt, and tetrabutylammonium hydrogen sulfate were from Merck (Darmstadt, Germany). Reference standards of albendazole sulfoxide, albendazole 2-aminosulfone, and albendazole sulfone were kindly donated by Pfizer Hellas (Athens, Greece). The albendazole sulfoxide standard used in this study was found to contain albendazole sulfone at a level of 4.98%; therefore, the concentrations of the standard solutions were appropriately corrected in all calculations to compensate for this impurity.

Standard Solutions. Stock standard solutions were prepared in individual 50 mL volumetric flasks by dissolving ca. 10 mg of each reference standard in 15 mL of dimethyl sulfoxide and diluting to volume with acetonitrile.

Mixed standard intermediate solution, containing all three analytes, was prepared by combining 10 mL of the albendazole sulfoxide and 0.5 mL of the albendazole 2-aminosulfone stock solutions in a 50 mL volumetric flask and diluting to volume with acetonitrile. The albendazole sulfone concentration of this intermediate solution was estimated taking into account that this compound constitutes 4.98% of the albendazole sulfoxide reference standard.

Concentrated mixed standard working solution was prepared by transferring ca. 0.5 mL of the mixed standard intermediate solution into a 25 mL volumetric flask, evaporating to dryness under N_2 at 40 °C, and dissolving to volume with mobile phase.

Mixed standard working solutions in the range of 12.3−307.2 ng/mL for albendazole sulfoxide, 0.4−11.1 ng/mL for albendazole 2-aminosulfone, and 0.16−3.84 ng/mL for albendazole sulfone were prepared by appropriate dilutions of the concentrated mixed standard working solution with mobile phase.

Mixed spiking solutions were prepared by transferring ca. 0.5 mL of the mixed standard intermediate solution into volumetric flasks (5, 10, and 25 mL) and appropriately diluting with acetonitrile.

Stock and mixed standard intermediate solutions were stable for at least 6 months in a freezer at −20 °C. Mixed standard working solutions were prepared weekly and were stored in a refrigerator when not in use.

Sample Extraction and Cleanup. An aliquot (1.5 g) of finely ground frozen tissue was homogenized for 30 s in a centrifuge tube with 6 mL of acetonitrile (for fat samples, a volume of 20 mL of isooctane was additionally introduced into the tube) using an Ultra Turrax. Following centrifugation at 4000*g* for 1 min, 1 mL of the clear acetonitrile layer was transferred to another tube and defatted with 3 mL of isooctane under vortexing for 15 s. After centrifugation at 2000*g* for 1 min, the isooctane layer was decanted, and 1 mL of 40 mM octanesulfonate solution in 0.04 M phosphoric acid and 3 mL of dichloromethane were added. The mixture was vortexed for 30 s and centrifuged at 2000*g* for 1 min. The bottom organic layer was transferred into another tube and evaporated to dryness under nitrogen at 40 °C. The residue was reconstituted in 1 mL of 0.02 M phosphate buffer, pH 8.5, and extracted with 5 mL of ethyl acetate under vortexing for 30 s. Following centrifugation at 2000*g* for 1 min, 4 mL of the top organic layer was transferred into another tube and evaporated to dryness under nitrogen at 40 °C. The residue was reconstituted in 0.5 mL of mobile phase, and 20 μL was submitted to chromatographic analysis.

LC Conditions. Analyses of extracted tissue samples and standard solutions were conducted using an acetonitrile−0.01 M *ortho*-phosphoric acid, (25:75, v/v) mobile phase that contained 5 mM octane sulfonate and 2.5 mM tetrabutylammonium ion pair reagents. Following its preparation, the mobile phase was passed through a 0.2 μm Nylon-66 filter (Anachem, Luton, United Kingdom) and degassed using helium. The mobile phase was delivered in the system at a rate of 1 mL/min.

The Nucleosil 100-5 C_{18} stationary phase was thoroughly equilibrated with the mobile phase, at ambient temperature, each time before use. Reproducible capacity factors could be realized after passage through the column of at least 150 mL of the mobile phase. Detection was made with excitation and emission wavelengths set at 290 and 320 nm, respectively; the sensitivity of the detector was set at "high"; and the response time was set at 1.5 s. The chart speed of the recorder was set at 5 mm/min.

Determination. Calibration curves were generated by analyzing mixed standard working solutions, plotting the recorded peak heights vs the corresponding masses of the analytes injected, and computing slope, intercept, and least-squares fits of standard curves. Slope and intercept data of calibration curves were used to compute the mass of the analytes in the 20 μL injected extracts. The concentration of each analyte in tissue samples was calculated by multiplying with the appropriate dilution and recovery factors.

Method Validation. For accuracy and precision studies, 1.5 g of aliquots of finely ground control sheep tissue (muscle, fat, liver, and kidney) were accurately weighed in 15 mL centrifuge tubes. Samples were thawed at room temperature and fortified at the desired concentration level by transferring portions of the mixed spiking solution. Fortified samples were allowed to stand for 30 min prior to extraction. For testing the method with real samples, albendazole-incurred tissue samples were also obtained from two sheep administered per os an albendazole drench 2.5% formulation.

RESULTS AND DISCUSSION

Sample Extraction and Cleanup. The trend in residue monitoring programs is the development of simple and cost effective isolation procedures that minimize time, labor, and consumables. In literature methods, the low part per billion levels at which benzimidazole residues should be detected in animal tissues have necessitated the development of costly and labor intensive multistep procedures for selective extraction of the analytes and removal of coextractants (*6*, *8*, *12*). Some workers have tried to overcome these problems by applying a simple and cost effective liquid−liquid partition procedure for the extraction and cleanup of albendazole residues from fish muscle (*11*). Unfortunately, when applying this procedure to sheep liver and kidney tissues, we encountered severe matrix interferences.

The sample preparation procedure described in this study is based on a simple liquid−liquid partition. Initial experiments on muscle and liver tissue samples showed that extraction and cleanup using one-step or two-step procedures previously described for milk (*13*−*15*) or cheese (*16*) analysis, respectively, failed to eliminate matrix interferences. Further investigation toward the possible use of other extraction solvents (dichloromethane, chloroform) resulted in the formation of severe emulsions and poor recoveries, especially for the 2-aminosulfone metabolite. To overcome these difficulties, a three-step extraction procedure was finally evaluated. Primary treatment of samples with four volumes of acetonitrile could effectively precipitate tissue proteins, but for fat samples, the addition of isooctane was necessary to dissolve fat and facilitate residues extraction into acetonitrile. The introduction of an isooctane washing of the acetonitrile extract removed many coextracted lipids. Further purification was made possible by enhancing ionization of albendazole metabolites at acidic conditions, adding

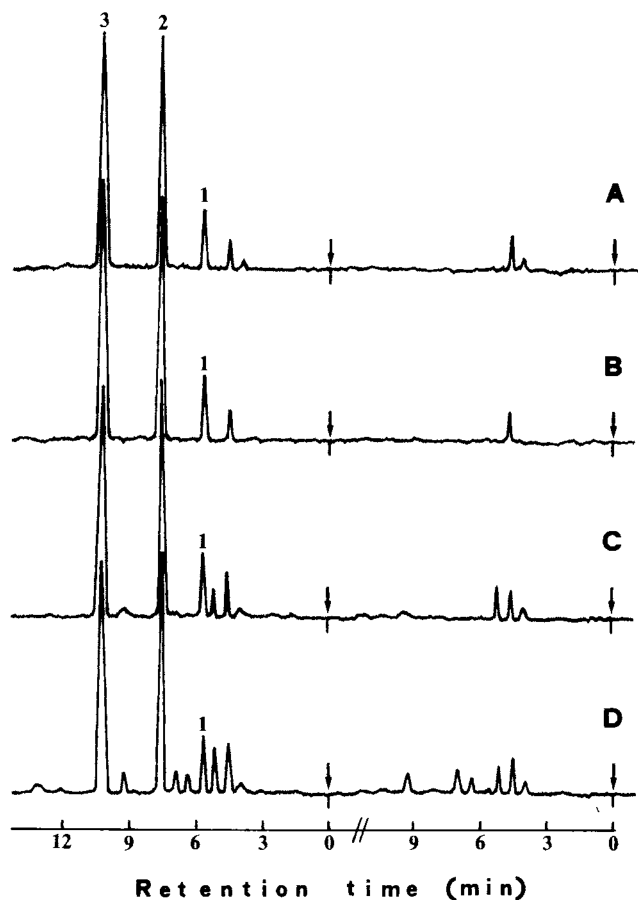

Figure 1. Chromatograms of control and spiked sheep muscle (**A**), fat (**B**), liver (**C**), and kidney (**D**) tissues. Peak identification: albendazole sulfoxide (1), albendazole 2-aminosulfone (2), and albendazole sulfone (3).

octanesulfonate pairing ions, and extracting formed ion pairs into dichloromethane. This ion pair extraction procedure proved to be critical for the efficient purification of tissue samples. Finally, partition of albendazole residues between ethyl acetate and a pH 8.5 phosphate buffer resulted in quite clean extracts. This simple and cost effective three-step extraction procedure resulted in quite clean extracts without causing any significant adverse effect on the recoveries of the albendazole metabolites.

LC Analysis. The previously described ionization-enhancing mobile phases that contained positively charged pairing ions (*13*, *14*) and those containing both positively and negatively charged pairing ions (*15*, *16*) resulted in poor resolution of the sulfoxide and the 2-aminosulfone metabolite from fluorescent matrix interferences originating from liver and kidney tissue samples. To improve the resolution while maintaining an acceptable degree of separation among all analytes, various modifications of the liquid chromatographic partitioning process, through addition to the mobile phase of different amounts of pairing ions, were investigated (*17*). The best results on separation and resolution could be obtained when the mobile phase contained 2.5 mM tetrabutylammonium hydrogen sulfate and 5 mM octanesulfonate sodium salt. Under these conditions, albendazole sulfoxide eluted at 5.7 min, albendazole 2-aminosulfone eluted at 7.6 min, and albendazole sulfone eluted at 10.2 min (**Figure 1**).

Calibration Curves. Regression analysis of the data obtained by running a series of working solutions showed that the response for all three analytes was linear in the range examined (0.246−6.144 ng of albendazole sulfoxide injected, 0.008−0.222 ng of albendazole 2-aminosulfone injected, and 0.003−0.077 ng of albendazole sulfone injected). Calibration curves that were based on eight data points with three replicates at each point could be described by the following equations: $y = 0.62 + 22.77x$, $r^2 = 0.9999$, for albendazole sulfoxide; $y = 0.97 + 729.8x$, $r^2 = 0.9996$, for albendazole 2-aminosulfone; and $y = 0.87 + 1664.9x$, $r^2 = 0.9994$, for albendazole sulfone, where y represents the peak height in millimeters and x is the quantity in nanograms of each analyte per 20 μL injected. The slopes of these calibration curves clearly showed the great sensitivity of the fluorescence detector toward both the 2-aminosulfone and the sulfone metabolites in contrast to albendazole sulfoxide.

System Suitability. The suitability of the LC system was checked with every set of 10 samples by running control and fortified tissue extracts and a complete set of mixed standard working solutions. The LC system provides correlation coefficients for standard curves better than 0.999 and baseline resolution and stable retention times of the albendazole metabolites, with $<$0.1 min change in retention times between runs.

The precision of the injection system was evaluated for the test compounds by injecting the calibration solutions of two concentration levels 10 times each on a single occasion. For each concentration, the relative standard deviation of the peak response was calculated. The results showed an adequately precise injection system with relative standard deviations of the peak heights better than 0.74% for all analytes at low concentration levels, while at higher levels, values better than 0.56% were determined.

The method specificity was also checked to ensure that there was no interference with the test compounds from matrix coextractives. Chromatograms obtained from control tissue extracts and reagent blanks showed that the peaks attributable to the test compounds were resolved sufficiently from any other peaks (baseline resolution) to enable reliable quantification (**Figure 1**).

Accuracy, Linearity, and Precision. The accuracy of the method was studied by spiking control sheep tissues (muscle, fat, liver, and kidney) with albendazole sulfoxide, albendazole 2-aminosulfone, and albendazole sulfone at five fortification levels and analyzing five replicates for each level. **Table 1** presents the fortification levels for each tissue and the individual mean recoveries found at each level for each analyte. Least squares and regression analysis of these data showed that the relationship between amounts added and amounts found could be adequately described by linear regressions (**Table 2**). The practically zero intercept values and the excellent linearity of the regression lines obtained permit the estimation of the overall recovery of the method on the basis of the slope of the corresponding regression line.

The precision of the method was studied by spiking control sheep tissues (muscle, fat, liver, and kidney) with standard analytes, at a certain concentration level for each compound, and analyzing five replicates, in three different days. The results are presented in **Table 3**.

Sensitivity. The efficiency of the ion pair chromatographic system coupled with the cleanliness of the extracts and the selectivity and high sensitivity of the fluorescence detector, particularly for the 2-aminosulfone and sulfone metabolites, allowed very low limits of detection to be realized in all target tissues. The limits of detection, defined as the average response determined in separate extractions from 20 different control tissue samples plus three times the standard deviation of the mean, are presented in **Table 4**.

Table 1. Accuracy Data for the Determination of Albendazole Residues in Animal Tissues

residue	fortification level (ng/g)	mean recovery[a] ± SD (%)			
		muscle	fat	liver	kidney
albendazole sulfoxide	20	98.9 ± 9.4	86.2 ± 5.1	102.8 ± 4.8	97.5 ± 7.6
	40	87.7 ± 4.0	87.9 ± 5.6	105.9 ± 9.3	88.9 ± 5.7
	80	87.6 ± 2.2	87.0 ± 5.5	94.8 ± 3.6	93.6 ± 7.5
	200	87.1 ± 4.0	84.8 ± 2.0	94.4 ± 4.4	98.6 ± 3.8
	1280	88.5 ± 3.6	81.6 ± 2.6	91.3 ± 3.0	91.3 ± 4.0
albendazole 2-aminosulfone	1	94.7 ± 6.9	85.5 ± 6.5	100.9 ± 8.4	93.6 ± 8.4
	4	93.7 ± 4.1	79.1 ± 2.8	94.9 ± 5.0	87.1 ± 4.8
	8	95.8 ± 3.5	81.9 ± 4.0	83.4 ± 4.3	91.9 ± 2.3
	20	88.5 ± 2.4	77.5 ± 2.3	83.1 ± 3.5	85.1 ± 1.8
	826	90.2 ± 3.2	76.8 ± 2.3	90.8 ± 3.8	93.8 ± 2.7
albendazole sulfone	0.5	107.5 ± 6.9	96.1 ± 7.3	109.3 ± 9.2	107.3 ± 7.7
	2	109.0 ± 1.7	92.0 ± 3.8	108.0 ± 4.4	109.6 ± 7.0
	4	102.3 ± 2.7	100.0 ± 3.3	104.5 ± 8.1	104.5 ± 5.2
	10	98.2 ± 3.4	93.5 ± 2.5	98.5 ± 3.9	94.0 ± 1.7
	64	101.9 ± 4.9	88.5 ± 3.6	100.7 ± 2.0	100.4 ± 2.3

[a] Mean of five replicates.

Table 2. Linearity Data for the Determination of Albendazole Residues in Animal Tissues[a]

residue	tissue	fortification range (ng/g)	slope	intercept	correlation coefficient	overall recovery ± SD (%)
albendazole sulfoxide	muscle	20–1280	0.884	–0.31	0.9985	88.4 ± 6.8
	fat	20–1280	0.814	1.61	0.9990	81.4 ± 4.6
	liver	20–1280	0.909	1.85	0.9989	90.9 ± 7.6
	kidney	20–1280	0.911	2.06	0.9980	91.1 ± 6.6
albendazole 2-aminosulfone	muscle	1–826	0.901	0.07	0.9990	90.1 ± 4.9
	fat	1–826	0.768	0.18	0.9993	76.8 ± 4.9
	liver	1–826	0.908	–0.46	0.9986	90.8 ± 8.8
	kidney	1–826	0.938	–0.54	0.9993	93.8 ± 5.6
albendazole sulfone	muscle	0.5–64	1.018	–0.03	0.9979	101.8 ± 5.7
	fat	0.5–64	0.882	0.27	0.9984	88.2 ± 5.5
	liver	0.5–64	1.005	0.05	0.9995	100.5 ± 7.7
	kidney	0.5–64	1.002	–0.02	0.9993	100.2 ± 8.2

[a] Each line represents five data points with five replicates at each data point.

Table 3. Precision Data for the Determination at Three Different Days of Albendazole Residues in Animal Tissues Spiked with Albendazole Sulfoxide (80 ng/g), Albendazole 2-Aminosulfone (8 ng/g), and Albendazole Sulfone (4 ng/g)

residue	tissue	mean concentration found[a] ± SD (ng/g)			RSD (%)			overall RSD (%)
		day 1	day 2	day 3	day 1	day 2	day 3	
albendazole sulfoxide	muscle	70.0 ± 1.7	72.4 ± 3.1	73.7 ± 2.7	2.5	4.4	3.7	4.0
	fat	69.6 ± 4.4	67.4 ± 3.7	70.1 ± 4.0	6.3	5.6	5.7	5.7
	liver	75.8 ± 2.8	72.9 ± 2.3	76.3 ± 5.1	3.8	3.2	6.7	5.0
	kidney	76.1 ± 3.8	78.7 ± 4.0	80.5 ± 3.8	5.0	5.1	4.8	5.1
albendazole 2-aminosulfone	muscle	7.6 ± 0.2	7.8 ± 0.2	7.5 ± 0.2	3.7	3.0	3.2	3.5
	fat	6.6 ± 0.3	6.2 ± 0.2	6.4 ± 0.3	4.9	3.3	4.8	4.9
	liver	6.6 ± 0.3	7.2 ± 0.3	6.9 ± 0.2	5.2	5.2	4.0	5.5
	kidney	7.3 ± 0.1	7.4 ± 0.3	7.7 ± 0.2	2.5	4.6	3.1	3.8
albendazole sulfone	muscle	4.0 ± 0.1	3.9 ± 0.1	4.1 ± 0.1	2.7	4.3	3.5	3.8
	fat	4.0 ± 0.1	3.8 ± 0.2	3.8 ± 0.2	3.3	4.3	5.1	4.9
	liver	4.1 ± 0.3	4.0 ± 0.2	4.4 ± 0.2	7.8	6.2	6.3	7.3
	kidney	4.1 ± 0.2	4.4 ± 0.1	4.4 ± 0.1	5.0	3.6	3.8	4.7

[a] Mean of five replicates.

The method of analysis was evaluated at the limit of quantification, defined as the lowest concentration of the test compound in tissues that has a precision (expressed as relative standard deviation) below or equal to 20% and a mean recovery within the range of 70−110%. To determine the limits of quantification, analysis was carried out on a series of five control tissue samples spiked at two different concentrations for each analyte. Analysis results showed that the limits of quantification of the method are those presented in **Table 4**.

Table 4. Detection and Quantification Limits (ng/g) of Albendazole Residues in Different Tissues

tissue	albendazole sulfoxide		albendazole 2-aminosulfone		albendazole sulfone	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
muscle	11.6	20.0	0.363	1.0	0.116	0.5
fat	6.3	20.0	0.197	1.0	0.099	0.5
liver	13.6	20.0	0.295	1.0	0.124	0.5
kidney	12.3	20.0	0.301	1.0	0.145	0.5

Interference Test. Because other veterinary drugs that are frequently administered to food animals might interfere with the analysis, an interference test was evaluated. Several compounds, such as oxytetracycline, tetracycline, chlortetracycline, penicillin G, cloxacillin, ampicillin, streptomycin, gentamicin, neomycin, thiabendazole, thiabendazole hydroxide, triclabenda-

Table 5. Albendazole Residues[a] in Tissues (ng/g) of Two Sheep Administered Per Os an Albendazole Formulation

tissue	albendazole sulfoxide 2 h	albendazole sulfoxide 7 days	albendazole 2-aminosulfone 2 h	albendazole 2-aminosulfone 7 days	albendazole sulfone 2 h	albendazole sulfone 7 days
muscle	354.2	<20.0	<1.0	<1.0	102.6	<0.5
fat	139.1	<20.0	<1.0	<1.0	50.3	<0.5
liver	1144.0	<20.0	4.2	1.7	682.6	1.2
kidney	215.0	<20.0	2.9	<1.0	399.6	<0.5

[a] Values are not corrected for recovery.

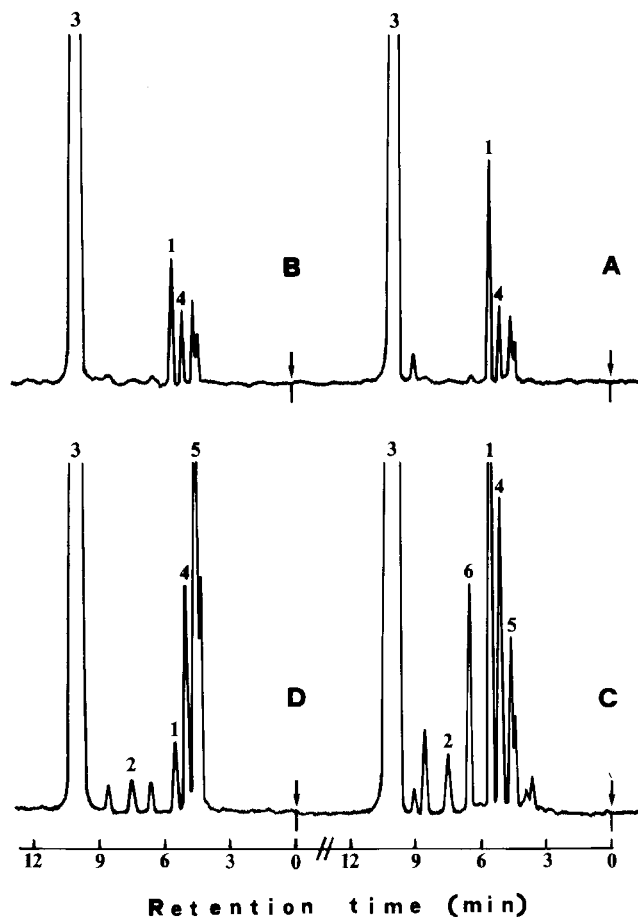

Figure 2. Chromatograms of incurred muscle (**A**), fat (**B**), liver (**C**), and kidney (**D**) tissues collected from a sheep 2 h after administration of an albendazole drench 2.5% formulation. Peak identification: albendazole sulfoxide (1), albendazole 2-aminosulfone (2), albendazole sulfone (3), and unknown metabolites (4–6).

zole, mebendazole, oxibendazole, febendazole, oxfendazole, febendazole sulfone, febendazole hydroxide, and febantel, were added to control muscle samples at 200 ng/g, and all samples were submitted to analysis. Results showed that none of the tested compounds interfered with the analysis.

Applicability. To validate the method with real samples, a trial was undertaken to quantitate residues in the tissues of two 8 month old healthy sheep, orally administered with a single oral dose of an albendazole drench 2.5% formulation corresponding to 15 mg of albendazole per kg of body weight. Samples of muscle, fat, liver, and kidney were taken, after the sheep were sacrificed (2 h and 7 days postdosing) and were submitted to analysis within the same day.

Residues of albendazole sulfoxide and albendazole sulfone were detected in all four tissues collected 2 h after the single oral administration while at the same time, albendazole 2-aminosulfone was detected only in liver and kidney tissue samples (**Table 5**). At day 7 postdosing, albendazole sulfoxide was not detected in any tissue sample, whereas albendazole 2-aminosulfone and albendazole sulfone were found only in liver.

Three unknown fluorescent compounds with retention times of 4.4, 5.0, and 6.5 min were detected in the liver tissue sample (**Figure 2C**), with the first two compounds also appearing in the chromatograms of the kidney tissue sample (**Figure 2D**), collected 2 h after albendazole administration. Furthermore, the second eluted unknown compound was also found in muscle (**Figure 2A**) and fat (**Figure 2B**) tissue samples, collected 2 h after albendazole administration. These fluorescent compounds could very possibly be unknown albendazole metabolites for the confirmation of which a liquid chromatographic and tandem mass spectrometric method described by Balizs (*12*) could prove to be quite useful.

The applicability of the method was also tested in swine and bovine tissues, which were fortified with albendazole metabolites. Because of the good analytical characteristics of the method, no matrix interferences were observed and the recovery values found were well within those already stated in the accuracy experiment.

In conclusion, the new method needs a very small sample size; offers considerable savings in terms of solvent requirements, costly materials, and sample manipulation; and presents satisfactory analytical characteristics with respect to recovery, sensitivity, selectivity, and reproducibility. Sample throughput (extraction/cleanup/evaporation/LC determination) is 12 samples in a total time of about 4 h by a single analyst. Because of these advantages, the method might be considered suitable for routine monitoring as well as for depletion studies of albendazole residues in animal tissues.

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