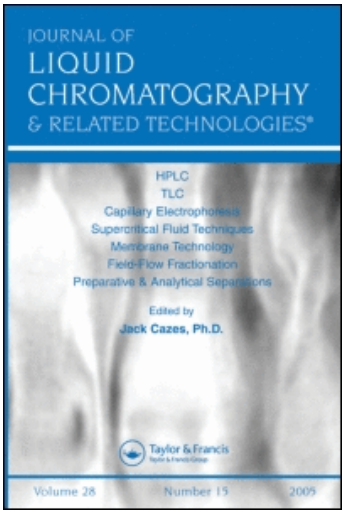

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ION-PAIR LIQUID CHROMATOGRAPHIC DETERMINATION OF ALBENDAZOLE IN CATTLE FEED

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ABSTRACT

A liquid chromatographic method is described for the determination of albendazole in cattle feeds. Samples are extracted with acetonitrile, and the extracts, after partitioning with hexane, are analyzed by ion-pair liquid chromatography with detection at 292 nm. Albendazole is eluted from the column well resolved from its oxidation byproducts. The method shows analytical characteristics well within acceptable limits. Recovery was in the range of 98.2-100.4%, while precision, expressed as percent relative standard deviation, in the range of 1.6-3.1%. Limit of detection was estimated at 0.5 μg/g, and limit of determination at 1.0 μg/g.

INTRODUCTION

Albendazole has proven to be an efficient and effective broad-spectrum anthelminthic in a variety of animal species, especially cattle,[1-3] and is marketed in a number of formulations for this purpose. It is available in suspension, bolus, and sustained-release formulations.[4] In addition, it is also formulated as a 20% premix which is intended for further dilution with feed to give albendazole-medicated feeds that could provide the drug to animals at 10 mg/kg body weight.[5]

Medication by feed is of interest because animals such as feedlot cattle could be mass-medicated by this route more economically rather than by individual treatment, whereas animals such as range cattle might be difficult to treat individually, or they might not be easily gathered during the optimal time for fluke control.[5-7]

As a result of this use, an analytical procedure capable to determine the dosage and to ensure homogeneity as well as stability of albendazole in cattle feed would be of value. A literature survey shows, however, that such methodology has never been reported. In contrast, several liquid chromatographic methods for assaying residues of albendazole and its metabolites in tissues and milk, as a result of cattle medication, have already been developed[8-12] in order to meet regulatory requirements for these teratogenic substances.[13] The objective of this work was to bridge this gap through development of a rapid, reliable, and stability-indicating method for the quantification of albendazole in cattle feed.

EXPERIMENTAL

Instrumentation

Liquid chromatography was carried out with a Gilson system (Gilson Medical Electronics, Villiers-le-Bel, France) consisting of a Model 805 manometric module, a Model 305 piston pump, a Model TC 831 column heater, and a Model 119 UV-vis detector. Injections were made using a Rheodyne, Model 7125, injection valve (Cotati, CA) equipped with 20-μL sample loop. Recordings were made with a Kipp & Zonen, Model BD 111, pen recorder (Delft, Holland).

A Model G-560E vortex mixer (Scientific Industries, Bohemia, NY), a Centra-MP4 centrifuge (IEC, Needman Heights, MA), and a Model ReactiTherm heating/stirring thermoblock (Pierce Chem., Rockford, IL), were used for sample treatment. A Model D7402 EasyPure UV compact ultrapure water system (Barnstead/Thermolyne Corp., Dubuque, IA) was also used for purification of tap water. To minimize drug adsorption, all needed glassware were pre-silanized prior use by treatment with 5% dichlorodimethylsilane (Sigma, St. Louis, MO) in toluene.

Reagents

Analytical grade hexane, phosphoric acid and tetrabutylammonium hydrogen sulfate, and HPLC grade acetonitrile were from Merck (Darmstadt, Germany). Standard albendazole, albendazole sulphoxide, and albendazole sulfone were from SmithKline (West Chester, PA).

Stock solutions of albendazole were prepared in 25-mL volumetric flasks by weighing ca 5 mg of the standard and diluting to the volume with acetonitrile. Intermediate solutions were also prepared by diluting aliquots from stock solution with acetonitrile. Working solutions in the range of 0.25 to 5.0 μg/mL were prepared by evaporating under N_2 at 40°C aliquots from the intermediate solution and diluting successively with mobile phase. To avoid photodegradation,[14] standards were protected from sunlight during handling and kept refrigerated when not in use.

Extraction/Cleanup Procedure

Feed sample was pulverized in a grinding mill (1 mm sieve screen) until uniform powder is obtained. A 2-g aliquot was weighed into a 50 mL screw-capped centrifuge tube, and 40 mL of acetonitrile were added. The tube was vortexed at high speed for 1 min and centrifuged for 2 min at 2000g. An aliquot (0.1-2.0 mL) of the clear supernatant, its volume depending on the expected level of albendazole in the analyzed sample, was transferred into another tube, and acetonitrile was added to a final volume of 2 mL.

Following volume adjustment, 4 mL of hexane were added, and the tube was vortexed for 15 s, and centrifuged. The top layer was discarded, and the remaining was evaporated to dryness under N_2 at 40°C. The residue was reconstituted in 0.5 mL of mobile phase, and an aliquot of 20 μL was submitted to liquid chromatographic analysis.

Liquid Chromatographic Analysis

Analysis was performed at 50°C on a reversed-phase Hichrom column, 250 x 4.6 mm, packed with Nucleosil 120 C_{18}, 5 μm, whereas a mobile phase consisting of acetonitrile/0.01 M phosphoric acid (20:80, v/v) and containing 5 mM tetrabutylammonium hydrogen sulfate was used to elute, isocratically, the analyte. The mobile phase was passed through 0.45 μm filter before use, degassed using helium, and delivered at a rate of 1.5 mL/min.

The Nucleosil 120 C_{18} stationary phase was thoroughly equilibrated with mobile phase each time before use. Reproducible capacity factors (k') could be realized after passage through the column of at least 150 mL of mobile phase.

After use, successive column washings with at least 200-mL volumes of water and acetonitrile were quite indispensable for removing the adsorbed pairing ions. Detection was made at 292 nm, whereas recordings at a chart speed of 5 mm/min and a sensitivity setting of 0.02 a.u.f.s.

Determination

Calibration curve was generated by running standard working solutions, plotting the recorded peak heights y (mm) versus the amounts x (ng), of albendazole injected, and computing the slope (b), intercept (a), and least square fit of the calibration curve according to the equation $y = a + bx$. Calibration curve slope and intercept data were used to determine the amount of the analyte in injected extracts. The concentration (μg/g) of the analyte in the feed sample was determined by multiplying the amount of the analyte by the appropriate dilution factor.

RESULTS AND DISCUSSION

Extraction/Cleanup

Animal feeds, in general, are very complex matrixes including a wide variety of organic compounds such as flavonoids, carotenoids, xanthophylls, and other pigments that usually occur in large amounts and could, therefore, interfere with the analysis of exogenous compounds such as the added drugs. The main key in performing a quantitative determination of a drug in a feed is the separation and removal of such interference.

Initial results of the assay procedure showed that extraction with dichloromethane or ethyl acetate using either a vortex mixer or an ultra-turrax homogenizer, failed to achieve complete recovery of albendazole. Since a possible reason for this incomplete recovery was irreversible adsorption of albendazole to the feed matrix, use of acetonitrile, an organic solvent with known de-binding ability,[14] was examined instead. Results showed that the use of acetonitrile in the extraction process could provide complete recovery of albendazole from the feed matrix.

Following extraction, feed extracts should be evaporated to dryness and reconstituted in mobile phase to become suitable for injection into the liquid chromatograph. This process, however, resulted in emulsions which were attributed to decreased dissolving power for the co-extracted feed lipids of the mobile phase as compared to the acetonitrile extractant. Experiments showed that a single partitioning of the feed extracts with hexane, prior to their evaporation, could eliminate the problem (Figure 1).

Liquid Chromatography

Albendazole, as a single analyte, is easily amenable to chromatography. Using literature mobile phases of acetonitrile/water or acetonitrile/phosphoric acid solutions, albendazole can be readily eluted from reversed-phase columns as a well-shaped peak.[15,16] However, difficulties can be encountered when co-

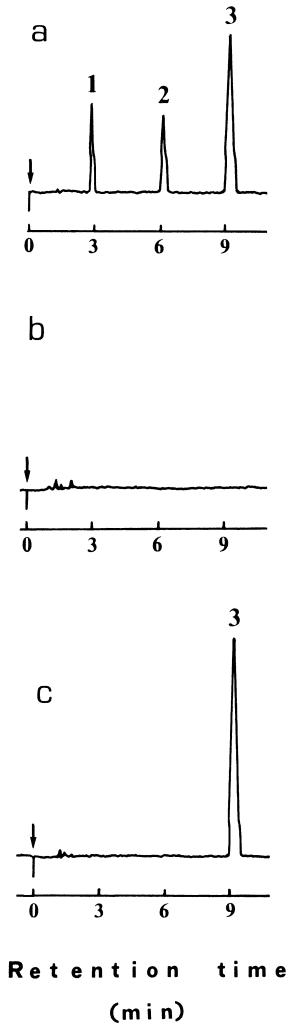

Figure 1. Typical chromatograms of a standard solution (a), a blank cattle feed sample (b), and a sample containing 200 μg/g of albendazole (c). Peak identification: albendazole sulfoxide (1), albendazole sulfone (2), and albendazole (3).

chromatography of albendazole with its oxidation products is attempted. When albendazole solutions are exposed to direct sunlight, several oxidation products have been shown to form. The degradation appears to proceed through stepwise oxidation at the sulfur atom leading to albendazole sulfoxide, albendazole sulfone and other more polar photolytic products.

Since such oxidation products might equally well form during storage of feeds medicated with albendazole, the chromatographic system suggested in this method should be capable of separating albendazole from known degradation products allowing, concurrently, their monitoring for stability-indicating purposes.

Previous work by other authors[9,10] has shown that mobile phases containing suitable pairing ions can be of help in separating mixtures of albendazole, albendazole sulfoxide, and albendazole sulfone. Using an acidic mobile phase containing 5 mM tetrabutylammonium reagent, peak resolution and symmetry can become excellent, peak heights can be enhanced, and all compounds can be chromatographed in reasonable time. This behavior has been attributed to efficient masking of the negatively charged silanols, and also to some electrostatic repulsion of the protonated analytes by the tetrabutylammonium cations adsorbed on to the octadecylsilica surface.[9] The chromatogram in Figure 1a demonstrates that these chromatographic conditions were capable of separating albendazole from known degradation products, proving that the method was also stability-indicating. Albendazole eluted from the column at 9.3 min, whereas albendazole sulfoxide and albendazole sulfone at 3 min and 6.2 min, respectively.

Accuracy and Precision

The accuracy and the precision of the method were studied by analyzing five sets of six 2-g feed samples, each set having previously been spiked with albendazole at 10, 50, 100, 200, and 400 μg/g levels, respectively (Figure 1b, c). Spiked samples were flushed with a gentle nitrogen stream for 1 min so that the spiking solvent would be evaporated, and then was allowed to stand for 1 h prior to analysis. Analysis results are summarized in Table 1. Quite acceptable recovery and variability values were noted at all spiking levels. Application of the method on albendazole-medicated cattle feeds revealed quite similar performance characteristics; triplicate analysis of a medicated feed with a claimed content of 100 μg/g showed a mean recovery of 98.5 ± 3.9%.

Linearity and Limits of Detection/Determination

The linearity of the absorbance response of the system with the amount of albendazole injected was evaluated in the range of 5-100 ng. Regression analysis of the data obtained by running six 20-μL triplicate injections corresponding to 5, 20, 40, 60, 80, and 100 ng of albendazole, respectively, showed the response to be linear in the range examined ($y = -0.02 + 1.22x$, $r = 0.99995$, where y represents peak height in mm and x the mass in ng of albendazole injected).

Table 1

Precision and Accuracy Data for the Determination of Albendazole in Cattle Feed

Albendazole Added, μg/g	Mean Concn. Found[a], μg/g	Rel. Std. Dev. %	Mean Recovery %
10.0	9.8 ± 0.3	3.1	98.3
50.0	50.2 ± 0.8	1.6	100.4
100.0	99.8 ± 2.3	2.3	99.8
200.0	196.4 ± 3.5	1.8	98.2
400.0	398.3 ± 8.4	2.1	99.6

[a] Mean of 6 replicates ± SD.

The power of the ion-pair chromatographic system coupled with the efficiency of the extraction/cleanup process, allowed reasonable limits of detection to be realized in medicated feeds. The limit of detection of albendazole in feeds, defined as the lowest level that could yield a signal clearly distinguished from the signals obtained by blank injections (3/1 ratio of peak height of the analyte to average peak-to-peak amplitude of the blank), was estimated at 0.5 μg/g.

To evaluate the limit of determination, a specific experiment was carried out. In this experiment, three sets of four 2-g feed samples, each set having previously spiked with albendazole at 0.5, 1.0 and 1.5 μg/g levels, respectively, were submitted to the whole analytical process. The limit of determination of albendazole in feeds, defined as the lowest level at which an acceptably precise estimate (RSD $\leq$ 10%) of the true concentration could be obtained, was estimated at 1.0 μg/g.

CONCLUSIONS

The developed method is a relatively simple, rapid, and inexpensive procedure that presents quite acceptable analytical characteristics with respect to recovery, precision, selectivity, and sensitivity. It offers the potential to control the homogeneity of medicated feeds, to certify the dosage level, to check the

stability status of the included albendazole, and to determine accidentally contaminated feeds. These advantages make the method valuable in the field of animal farming.

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