# Trace analysis of albendazole and its sulphoxide and sulphone metabolites in milk by liquid chromatography

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## Abstract

Analytical methodology for determination of albendazole and its sulphoxide and sulphone metabolites in milk at levels down to 2–5 ng/ml has been developed. Extraction was carried out with ethyl acetate under alkaline conditions, and extracts were analyzed on a silica-based $C_{18}$ column in the presence of positively-charged pairing ions. Accuracy data showed overall recoveries ranged from 78.4% to 100%, whereas precision data, based on within- and between-day variation, suggested overall precision values better than 4.9%. The method was successfully applied to determine residues in milk of a dairy cow orally given albendazole.

*Keywords:* Albendazole; Albendazole sulphoxide; Albendazole sulphone

## 1. Introduction

Albendazole is an anthelmintic benzimidazole which has shown efficacy against all classes of helminths that commonly infect animals. Its use has been approved in cattle [1] but is not recommended in dairy cattle of breeding age because the drug and its metabolites may manifest themselves as residues in milk, posing a health threat to consumers. Toxicological studies in both farm and laboratory animals have shown albendazole and its metabolites to be teratogenic [2]. In this regard, a maximum residue limit of 100 ng/ml for total albendazole residues has been proposed by regulatory agencies [3], whereas a number of analytical methods have been developed to ensure the safety of marketed milk. However,

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reported methods are confined to the determination of the parent compound [4,5] or its 2-aminosulphone metabolite [6]; adequate methodology for the extraction, separation and quantitation of the major sulphoxide and sulphone metabolites of albendazole in milk has not been yet described.

The aim of this study was to develop a simple, fast, inexpensive, sensitive and reliable method for determination of albendazole and its sulphoxide and sulphone metabolites in milk. This goal was achieved using liquid–liquid partition procedures for extraction and clean-up, and ion-pair liquid chromatography for efficient determination. The feasibility of using polymeric stationary phases for separation of the albendazole residues also was evaluated to overcome difficulties often encountered in reversed-phase chromatography of basic compounds. The method was successfully applied to determine alben-

dazole and its sulphoxide and sulphone metabolites in milk of a dairy cow orally given albendazole.

## 2. Experimental

### 2.1. Apparatus

Chromatography was carried out on a Gilson system (Villiers-le-Bel, France) consisting of a Model 805 manometric module, a Model 305 piston pump, a Model 119 UV–Vis detector, and a Model TC 831 column oven. Injections were made through a Rheodyne 7725 (Cotati, CA, USA) sample injector equipped with a 100-$\mu$l loop, whereas recordings were made using a Kipp and Zonen Model BD 111 (Delft, Netherlands) recorder. Two liquid chromatographic columns were tested in this study: a Hichrom (Reading, UK) column, 250×4.6 mm, packed with 5-$\mu$m Nucleosil 120 C$_{18}$, and a Hamilton PRP-1 (Reno, NE, USA) polymeric column, 150×4.1 mm, packed with 10-$\mu$m poly(styrene–divinylbenzene).

A vortex mixer Model G-560E (Scientific Industries, NY, USA), an IEC Model Centra-MP4 (Needman Heights, MA, USA) centrifuge, and a thermoblock Model ReactiTherm heating/stirring module (Pierce, Rockford, IL, USA) were also used in sample preparation.

### 2.2. Reagents

Analytical-grade octanesulfonic acid sodium salt and dichlorodimethylsilane were obtained from Sigma (St. Louis, MO, USA), while LC-grade acetonitrile, ethyl acetate, toluene and tetrabutylammonium hydrogen sulfate were purchased from Merck (Darmstadt, Germany). Standard albendazole, albendazole sulphoxide, and albendazole sulphone were all kindly donated by SmithKline Beecham (Veterin SA, Athens, Greece).

### 2.3. Standard solutions

Stock solutions of the individual benzimidazoles (ca. 100 $\mu$g/ml) were prepared in 50-ml volumetric flasks by dissolving ca. 5 mg of each compound and diluting to volume with acetonitrile. Intermediate and working solutions were prepared by successive dilu-

tion of stock solutions with mobile phase to give solutions in the range 16–320 ng/ml for albendazole, 4–85 ng/ml for albendazole sulphoxide, and 8–160 ng/ml for albendazole sulphone. To avoid photodegradation [7], standard solutions and milk extracts were protected from direct sunlight by carrying out all analytical process in a subdued-light laboratory.

### 2.4. Chromatographic conditions

The mobile phase was a mixture of acetonitrile and 0.01 $M$ phosphoric acid (20:80, v/v) containing 5 m$M$ tetrabutylammonium hydrogen sulfate. Following mixing, the mobile phase was passed through a 0.45-$\mu$m filter, and degassed using helium. The mobile phase was delivered in the system at a rate of 1.0 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. During runs the column was kept thermostated at 50°C. 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 0.005 AUFS.

### 2.5. Extraction and clean-up procedure

Both extraction and clean-up steps were performed in 15-ml screw-capped centrifuge tubes pre-treated with 5% (v/v) dichlorodimethylsilane in toluene in order to minimize drug adsorption. Milk sample (1.5 ml) was alkalinized to pH 9.8 by addition of 100 $\mu$l 0.4 $M$ sodium hydroxide solution, and extracted with 8 ml ethyl acetate under high speed vortexing for 30 s. After centrifugation at 4000 $g$ for 2 min, an aliquot (6 ml) of the clear supernatant was transferred to another tube, and 2 ml of water were also added. The mixture was vortexed for 10 s and centrifuged at 1000 $g$ for 30 s. Following centrifugation, the top organic layer was transferred into a tube, and evaporated to dryness under nitrogen at 40°C. The residue was reconstituted in 0.5 ml of mobile phase, filtered

through a 0.2-$\mu$m filter, and submitted to chromatographic analysis.

## 2.6. Determination

Calibration curves were generated by running a series of working solutions, plotting the recorded peak heights versus the corresponding mass of the analytes injected, and computing slope, intercept and least squared fit of standard curves. Quantitation of analytes in milk samples was realized by reference to corresponding standard curves, calculating the mass of the analytes, and multiplying with appropriate dilution and recovery factors.

## 3. Results and discussion

### 3.1. Extraction and clean-up

Polarity is usually the most important factor in choosing the extraction solvent. The closer the solvent polarity is to the analyte polarity, the higher the extraction efficiency may be. Considering that best solvent for a selective extraction procedure should be the most apolar solvent with which the analytes could be extracted with sufficient efficiency, ethyl acetate was chosen as the extraction solvent. Ethyl acetate was selected to extract albendazole and its sulphoxide and sulphone metabolites from milk samples not only because of its high solvating power for these weakly basic compounds (Fig. 1) but also for its ability to form emulsion-free interfaces below a certain sample/solvent volume ratio.

Apart from polarity, the extraction efficiency of ethyl acetate could be also determined by the pH of the sample/solvent system. Initial experiments on the partition of the analytes between ethyl acetate and various phosphate buffers in the pH range 3.1–11.4 showed that the compounds could be quantitatively partitioned into the organic solvent up to pH of 10.3; at higher pH, the partitioning of albendazole sulphoxide into the ethyl acetate was considerably decreased while that of albendazole and albendazole sulphone remained almost unchanged (Table 1). Further experimentation on fortified milk samples revealed a different partitioning profile. In acidified milk, the extraction efficiency of ethyl acetate,
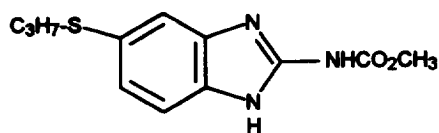


**Albendazole**
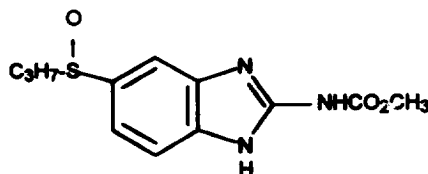
**Albendazole sulphoxide**
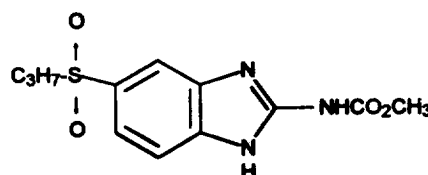
**Albendazole sulphone**

Fig. 1. Chemical structures of investigated benzimidazoles.

although satisfactory for both albendazole and albendazole sulphone, was poor for albendazole sulphoxide; however, it markedly improved with increasing pH to arrive at its maximum at pH 9.8 for the sulphoxide and sulphone metabolites, and at pH 11.4 for the parent drug. Considering that the partitioning of the sulphoxide metabolite into the ethyl acetate

Table 1
Efficiency of ethyl acetate in the extraction of albendazole, albendazole sulphoxide and albendazole sulphone from phosphate buffers and milk samples in relation to their pH value

| pH value | Extraction efficiency[a] of ethyl acetate, % | | | | | |
|---|---|---|---|---|---|---|
| | Albendazole | | Albendazole sulphoxide | | Albendazole sulphone | |
| | Buffer | Milk | Buffer | Milk | Buffer | Milk |
| 3.1 | 100 | 73 | 97 | 41 | 101 | 89 |
| 6.6 | 98 | 72 | 97 | 90 | 100 | 92 |
| 9.8 | 100 | 81 | 97 | 92 | 100 | 100 |
| 10.3 | 100 | 85 | 94 | 86 | 100 | 98 |
| 11.4 | 98 | 89 | 71 | 56 | 97 | 97 |

[a] Mean of three replicates.

was again considerably decreased above pH 9.8 (Table 1), this value was selected as the optimum extraction pH.

Analysis of blank milk without any clean-up processing of the ethyl acetate extracts resulted in chromatograms containing matrix peaks. Considering that these matrix compounds might be transferred into the ethyl acetate through the trace amount of water which this organic solvent can dissolve, water-saturated instead of pure ethyl acetate was tested as extractant but it had no effect. Thus, an effective washing step of the ethyl acetate extract with water was finally introduced although it had an adverse effect on the recovery of the sulphoxide metabolite.

## 3.2. Chromatography

Owing to their polar characteristics, albendazole and its sulphoxide and sulphone metabolites are not easily amenable to analysis with a single chromatographic system. Using silica-based $C_{18}$ or poly-(styrene–divinylbenzene) stationary phases and various neutral-pH mobile phases that have been described for the analysis of albendazole in biological fluids [8] and milk [5], and albendazole and albendazole sulphoxide in biological fluids [9,10], the parent compound could be readily chromatographed but its sulphoxide and sulphone metabolites had almost identical retention times. By changing to acidic mobile phases that have been used for the analysis of albendazole in milk [4] and tissues [11,12], separation of the sulphoxide and sulphone metabolites occurred but detection of the parent compound turned out to be problematic as it appeared as a broad and badly tailed peak. The extent of peak tailing differed with the type of the stationary phase, being more pronounced with the silica-based one. Because this indicated that a relatively strong adsorptive interaction between the protonated albendazole and negatively charged silanol groups on the silica-based stationary phase should exist, deactivation of active sites by addition to the mobile phase of tetrabutylammonium cations [13] was examined. Moreover, improvement of the chromatographic performance of the poly(styrene–divinylbenzene) stationary phase was attempted by enhancing the partition characteristics of albendazole through addition to the mobile phase of octanesulfonate anions

and/or tetrabutylammonium cations. Such pairing ions have been effectively used in chromatographic analysis of other benzimidazoles for eliminating peak tailing [14].

Following addition of 10 mM octanesulfonate anions to the mobile phase, the chromatographic performance of the poly(styrene–divinylbenzene) stationary phase improved; peak heights of all analytes enhanced, and the tailing of albendazole was eliminated although its retention significantly increased. Changing to a higher elution strength mobile phase, the elution time of albendazole could be shortened, but its metabolites eluted close each other and near the solvent front (Fig. 2).

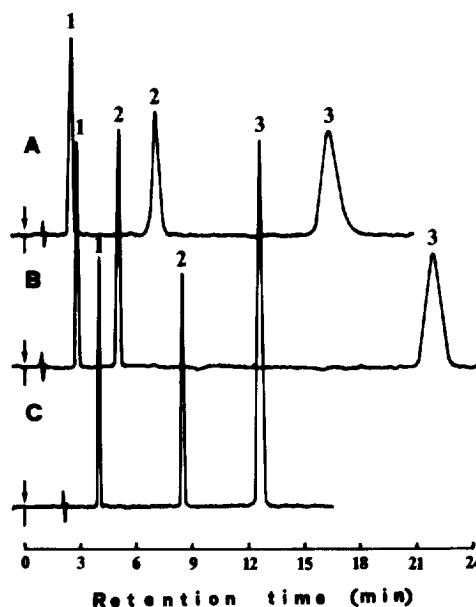Despite the substantial improvement of the polymeric stationary phase upon use of the ion pairing



Fig. 2. Chromatograms of a standard solution, which contained 50 ng/ml albendazole sulphoxide (1), 100 ng/ml albendazole sulphone (2) and 200 ng/ml albendazole (3), run with different stationary and mobile phases. (A) Stationary phase: Hamilton PRP-1; mobile phase: acetonitrile–0.01 M phosphoric acid (20/80, v/v). (B) Stationary phase: Hamilton PRP-1; mobile phase: acetonitrile–0.01 M phosphoric acid (28/72, v/v), containing 10 mM octanesulphonate anions. (C) Stationary phase: Nucleosil $C_{18}$; mobile phase: acetonitrile–0.01 M phosphoric acid (20/80, v/v), containing 5 mM tetrabutylammonium hydrogen sulfate. Other chromatographic conditions: column temperature, 50°C; flow-rate, 1 ml/min; wavelength, 292 nm; recorder sensitivity, 0.005 AUFS; chart speed, 5 mm/min; injection volume, 100 $\mu$l.

mixture, much better results could be obtained with the silica-based stationary phase when tetrabutylammonium cations were added to the mobile phase. Peak heights of all three analytes were greatly increased, peak distortion was totally eliminated, and peak resolution was excellent (Fig. 2C). This behavior may be attributed not only to efficient masking of the negatively charged silanols but also to some electrostatic repulsion of the protonated analytes by the tetrabutylammonium cations adsorbed on to the octadecylsilica surface.

Peak heights of all analytes, although quite reproducible at a given mobile phase flow-rate, varied with it. Increasing the flow-rate from 0.6 to 1.5 ml/min, peak heights of albendazole and albendazole sulphone decreased approximately 57% and 25%, respectively. Peak height of albendazole sulphoxide also showed a gradual decrease (19%) when flow-rate increased from 0.6 to 1.3 ml/min but thereafter (1.5 ml/min) remained constant (Fig. 3). Using the developed ion-pair chromatographic system, albendazole eluted from the column at 12.5 min whereas its sulphone and sulphoxide metabolites eluted at 8.5 min and 4.0 min, respectively.

The temperature of the column had also a remarkable effect on both the separation and the chromatographic efficiency. Increasing column temperature up to 50°C, a progressive reduction of the capacity factors occurred for both the albendazole and its sulphone metabolite but not for the early eluted sulphoxide metabolite. Increasing column temperature had also a beneficial effect on peak heights of all analytes, the effect being more pronounced for the late eluted albendazole and albendazole sulphone. At
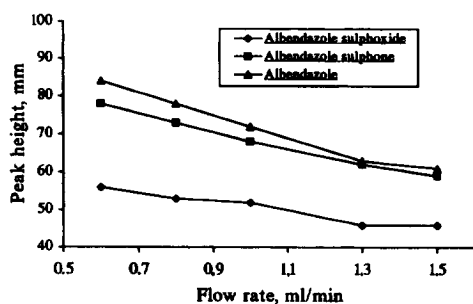
the temperature of 50°C, the shape of all peaks was substantially improved due possibly to better mass transfer of the compounds between the stationary and mobile phase, a finding suggesting that control of temperature may be of help in specific ion-pair separations.

### 3.3. Linearity and limit of detection

Regression analysis of the data obtained by running a series of working solutions showed the response to be linear in the range examined (1.6–32.0 ng of albendazole injected; 0.4–8.5 ng of albendazole sulphoxide injected; 0.8–16.0 ng of albendazole sulphone injected). Calibration curves could be described by the following equations: $y = -0.56 + (4.80 \pm 0.07)x$, $r = 0.99991$, for albendazole; $y = 0.75 + (12.96 \pm 0.17)x$, $r = 0.99994$, for albendazole sulphoxide; $y = 0.21 + (5.95 \pm 0.04)x$, $r = 0.99998$, for albendazole sulphone, where $y$ represents peak height in mm and $x$ the quantity in ng of the compound per 100 $\mu$l injected. The efficiency of the ion pair chromatographic system coupled with the cleanliness of the extracts, and the excellent response of the compounds at 292 nm could allow low limits of detection (5 ng/ml for albendazole, 2 ng/ml for albendazole sulphoxide, and 3 ng/ml for albendazole sulphone) to be realized (peak-to-noise ratio, 3/1).

### 3.4. Accuracy and precision

The accuracy of the method was studied by spiking milk samples with albendazole, albendazole sulphone and albendazole sulphoxide at 4 fortification levels and analyzing 5 replicates. Least-squares and regression analysis of the data presented in Table 2 showed that the relationship between "added" and "found" could be adequately described by a linear regression for each of the analytes: $y = 0.25 + (0.817 \pm 0.037)x$, $r = 0.99909$ for albendazole; $y = -0.29 + (0.784 \pm 0.041)x$, $r = 0.99880$ for albendazole sulphoxide; $y = -0.14 + (1.000 \pm 0.046)x$, $r = 0.99908$ for albendazole sulphone. Therefore, the slopes of these regression lines could be used as estimates of the overall recovery of the method for albendazole (81.7 ± 3.7%), albendazole sulphoxide (78.4 ± 4.1%) and albendazole sulphone (100 ± 4.6%).



Fig. 3. Peak heights of albendazole sulphoxide, albendazole sulphone and albendazole as a function of the mobile phase flow-rate.

Table 2
Accuracy data for the determination of albendazole, albendazole sulphoxide and albendazole sulphone residues in milk

| Concentration added (ng/ml) | Concentration found[a] (ng/ml) | R.S.D. (%) | Mean recovery (%) |
|---|---|---|---|
| *Albendazole* | | | |
| 21.3 | 17.6±0.5 | 2.8 | 82.6 |
| 42.7 | 35.4±0.7 | 1.9 | 82.8 |
| 64.0 | 52.2±0.7 | 1.4 | 81.6 |
| 85.3 | 70.1±1.2 | 1.7 | 82.1 |
| | | | |
| *Albendazole sulphone* | | | |
| 21.3 | 21.1±0.6 | 2.6 | 99.2 |
| 42.7 | 42.7±0.7 | 1.6 | 99.9 |
| 64.0 | 63.8±1.3 | 2.0 | 99.7 |
| 85.3 | 85.2±1.3 | 1.6 | 99.9 |
| | | | |
| *Albendazole sulphoxide* | | | |
| 22.7 | 17.4±0.8 | 4.5 | 76.4 |
| 45.3 | 35.5±0.7 | 1.8 | 78.4 |
| 68.0 | 52.9±1.1 | 2.2 | 77.8 |
| 90.7 | 70.8±1.2 | 1.7 | 78.1 |

[a] Mean of five samples±S.D.

The precision of the method was studied by assaying, on each of three different days, 4 milk samples spiked with 21.3 ng/ml of albendazole and 22.7 ng/ml of each albendazole sulphoxide and albendazole sulphone. To estimate the components of variance, the concentrations found (Table 3) were subjected to "analysis of variance and expected mean squares for the one way classification-balanced design" [15]. Analysis data showed that the within-day precision was better than the between-day precision for all analytes. The overall precision, which is in fact the overall uncertainty of a single determination, was estimated to be 3.0%, 4.9% and 2.3%, for albendazole, albendazole sulphoxide and albendazole sulphone, respectively.

*3.5. Interference test*

Since other drugs or antibiotics that are frequently administered to cattle might interfere with the analysis, an interference test was evaluated. Several compounds such as penicillin G, penicillin V, oxacillin, cloxacillin, ampicillin, amoxycillin, streptomycin, gentamicin, kanamycin, neomycin, gentamicin, chlortetracycline, tetracycline, oxytetracycline, thiabendazole, thiabendazole hydroxide, triclabendazole, mebendazole, oxibendazole, febendazole, oxfendazole, febendazole sulphone, febendazole hydroxide and febantel were added to milk samples at 0.2 μg/ml level and all samples were submitted to analysis. Results showed that none of the tested compounds interfered with the analysis.

*3.6. Applicability*

To validate the method with real samples, a trial was undertaken to quantitate residues in the milk of a lactating dairy cow, orally administered with a single dose of albendazole formulation (Albendazole 600, Veterin SA, Greece) corresponding to approximately 15 mg of albendazole per kg of body mass. The control milk sample, which was taken before treatment and all of the other samples collected during the trial at 12-h milking intervals were stored at −25°C, until analyzed (Table 4). The very low level of the parent albendazole in comparison with the high concentrations of its sulphoxide and sulphone metabolites in the sample obtained at the first milking are consistent with previous pharmacokinetic studies on cattle suggesting a rapid first-pass metabolism of the compound in the liver [2]. The albendazole sulphoxide metabolite, which is the product of the primary oxidation of the sulfide moiety of albendazole to sulphoxide, arrived at its maximum

Table 3
Precision data based on the analysis at three different days of four milk samples spiked with 21.3 ng/ml of albendazole and albendazole sulphone and 22.7 ng/ml of albendazole sulphoxide

| Day | Concentration found (ng/ml) | Mean concentration (ng/ml) | Variance estimates, R.S.D. (%) | |
|---|---|---|---|---|
| *Albendazole* | | | | |
| 1 | 17.1, 17.8, 18.3, 17.2 | 17.6±0.5 | 2.8 | |
| 2 | 16.9, 17.4, 17.4, 17.9 | 17.4±0.4 | 2.0 | |
| 3 | 17.0, 18.6, 17.5, 17.9 | 17.8±0.6 | 3.3 | |
| | | | Within-day | 3.2 |
| | | | Between-day | 2.0 |
| | | | Overall | 3.0 |
| *Albendazole sulphone* | | | | |
| 1 | 21.4, 20.3, 21.0, 21.8 | 21.1±0.6 | 2.6 | |
| 2 | 21.3, 21.5, 21.0, 22.0 | 21.5±0.4 | 1.7 | |
| 3 | 21.1, 20.9, 20.7, 21.6 | 21.1±0.3 | 1.6 | |
| | | | Within-day | 2.3 |
| | | | Between-day | 1.9 |
| | | | Overall | 2.3 |
| *Albendazole sulphoxide found* | | | | |
| 1 | 17.9, 16.2, 18.2, 17.1 | 17.4±0.8 | 4.5 | |
| 2 | 17.2, 16.0, 18.5, 18.0 | 17.4±0.9 | 5.4 | |
| 3 | 16.1, 17.9, 17.0, 17.5 | 17.1±0.7 | 3.9 | |
| | | | Within-day | 5.4 |
| | | | Between-day | 1.8 |
| | | | Overall | 4.9 |

12 h after dosing and declined below detectable levels by 48 h, while the sulphone metabolite, which is the end-product of the 2-step oxidation of albendazole, attained its highest level more slowly (24 h) and disappeared also more slowly (84 h).

Although albendazole sulphone could not be detected at a withdrawal time of 84 h or longer, another major polar metabolite, which was eluted at 2.8 min, could be seen by the 96 h milking (Fig. 4). This metabolite was present at low concentration in the milk obtained at the first milking and arrived at its maximum at the third milking (36 h after dosing). On the basis of standards, this metabolite was identified as the N-deacetylation product of albendazole sulphone, which is the 2-aminosulphone metabolite, but was not quantified. A less polar

Table 4
Residues of albendazole and its sulphoxide and sulphone metabolites in milk of a dairy cow administered orally an albendazole formulation

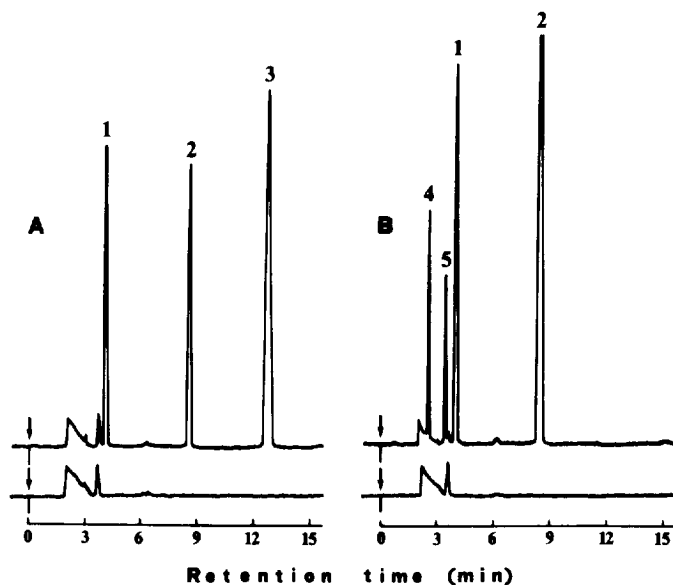| Time after administration (h) | Albendazole found[a] (ng/ml) | Albendazole sulphoxide found[a] (ng/ml) | Albendazole sulphone found[a] (ng/ml) |
|---|---|---|---|
| 12 | 5.2 | 800.0 | 853.3 |
| 24 | <5 | 163.2 | 931.2 |
| 36 | <5 | 3.5 | 26.7 |
| 48 | | <2 | 16.0 |
| 60 | | <2 | 6.4 |
| 72 | | | 3.3 |
| 84 | | | <3 |
| 96 | | | <3 |

[a] Values have not been corrected for recovery.

Fig. 4. Typical chromatograms of extracts from (A) blank control and milk fortified with 34.8 ng/ml albendazole sulphoxide, 56 ng/ml albendazole sulphone and 109 ng/ml albendazole and from (B) blank control and albendazole incurred milk. Peaks: albendazole sulphoxide (1), albendazole sulphone (2), albendazole (3), albendazole-2-aminosulphone (4), unknown metabolite (5). Chromatographic conditions as in Fig. 2C.

metabolite, which was eluted at 3.6 min unresolved from matrix interference, could be also observed but not identified. Its concentration was highest 12 h after dosing and declined below detection at the fifth milking.

## 4. Conclusion

The results of the present study show that the developed method is an efficient and reliable means for quantitating albendazole and its sulphoxide and sulphone metabolites in milk. The method has quite satisfactory analytical characteristics with respect to recovery, sensitivity, selectivity and reproducibility. Sample throughput (extraction/clean-up and chromatographic analysis) is 10 samples per 3 h for a single analyst. Owing to its simplicity and inexpensiveness, the method might be considered suitable to serve as a very rapid test for routine identification of albendazole contamination in milk.

## Acknowledgments

## References

[1] Code of Federal Regulations, 21CFR520.45, 1989.
[2] P. Delatour and R. Parish, in A.G. Rico (Editor), Drug Residues in Animals, Academic Press, Orlando, FL, 1986, p. 175.
[3] FAO/WHO Expert Committee. Evaluation of certain veterinary drug residues in food; Thirty-fourth report of the joint FAO/WHO Expert Committee on Food Additives; Technical Report Series 788; World Health Organization, Geneva, 1989.
[4] A.R. Long, L.C. Hsieh, M.S. Malbrough, C.R. Short and S.A. Barker, J. Assoc. Off. Anal. Chem., 72 (1989) 739.
[5] D.S. Nakos, N.A. Botsoglou and I.E. Psomas, J. Liq. Chromatogr., 17 (1994) 4145.
[6] P.-S. Chu, R.Y. Wang, T.A. Brandt and C.A. Weerasinghe, J. Chromatogr., 620 (1993) 129.

[7] C.A. Weerasinghe, D.O. Lewis, J.M. Mathews, A.R. Jeffcoat, P.M. Troxler and R.Y. Wang, J. Agric. Food Chem., 40 (1992) 1413.

[8] J.A. Bogan and S. Marriner, J. Pharm. Sci., 69 (1980) 41.

[9] A. Meulemans, M.D. Giovanangeli, J. Mohler, M. Vulpillat, J.M. Hay and A.G. Saimot, J. Liq. Chromatogr., 7, (1984) 569.

[10] M. Hurtado, M.T. Medina, J. Sotelo and H. Jung, J. Chromatogr., 494 (1989) 403.

[11] S. A Barker, T. McDowell, B. Charkhian, L.C. Hsieh and C.R. Short, J. Assoc. Off. Anal. Chem., 73 (1990) 21.

[12] A.R. Long, M.S. Malbrough, L.C. Hsieh, C.R. Short and S.A. Barker, J. Assoc. Off. Anal. Chem., 73 (1990) 860.

[13] N.A. Botsoglou, D.J. Fletouris, I.E. Psomas and V.N. Vassilopoulos, J. Liq. Chromatogr., 17 (1994) 4229.

[14] D.J. Fletouris, N.A. Botsoglou, I.E. Psomas and A.I. Mantis, Analyst, 119 (1994) 2801.

[15] G.T. Wernimont, in W. Spendley (Editor), Use of Statistics to Evaluate Analytical Methods; Association of Official Analytical Chemists, Arlington, VA, 1987.