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## LIQUID CHROMATOGRAPHIC DETERMINATION OF DIMETRIDAZOLE AND RONIDAZOLE IN FEEDS

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### ABSTRACT

A simple high performance liquid chromatographic (HPLC) procedure for the simultaneous determination of dimetridazole and ronidazole in turkey feeds is described. The drugs are extracted from feeds by carbon-tetrachloride/dimethylformamide (80:20) at 60°C during 30 mn and the extract is subjected to a partition by water. After centrifugation the eluate is chromatographed on a reverse phase column with ultraviolet detection at 316 nm. Recoveries from samples fortified at levels 2.02 to 7.07 ppm for ronidazole and 2.01 to 7.03 for dimetridazole were 99,6%  $\pm$  1,4 and 95,3%  $\pm$  1,8 (mean  $\pm$  standard deviation), respectively.

### INTRODUCTION

The prevention of coccidiosis, a disease caused by intestinal parasite of *Eimeria* class, obliges the poultry feed manufactures to include additives of the coccidiostat group in the feeds.

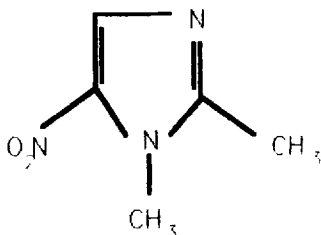


Figure 1 - Structural formula of dimetridazole

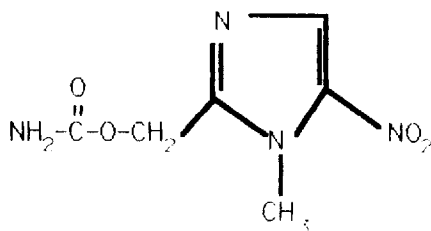


Figure 2 - Structural formula of ronidazole

Dimetridazole (1,2-dimethyl - 5 - nitroimidazole), figure 1, is an inexpensive and effective nitroimidazole which added to feeds, treats and prevents some diseases caused by anaerobic bacteria and protozoal parasites [1,2]. It's used for the treatment and prevention of histomoniasis (blackhead) in turkeys [2-8], of dysentery and trichomoniasis in swines [2,5,8,9], of trichomoniasis in pigeons and coccidiosis in rabbits [2]. Portuguese law allows this additive in turkey and guinea-fowl feeds in doses 100-200 mg/kg and 125-150 mg/kg [10], respectively

Ronidazole (1-methyl-5-nitroimidazol-2-ylmethyl carbamate), figure 2, is a nitroimidazole with antiparasitic activity, very useful in animal husbandry. It's used as additive in feeds with the aim of treat and/or prevent enterohepatitis [11] and histomoniasis [12] In turkeys and dysentery in swines [12,13]. In our country its addition, only allowed in turkey feeds, ranges from 60 to 90 mg/kg [10].

Over their antimicrobial effects, the nitroimidazoles have growth promoting properties [9].

The present work proposes a common extraction method for coccidiostats from turkey feeds and their simultaneous quantification by HPLC at the same wavelength. The method uses a high sensitive technique and it is innovator when compared with the method of the Association of Official Analytical Chemists [14] recommended for the extraction and quantification of dimetridazole in feeds.

## EXPERIMENTAL

### Reagents and apparatus

N,N - dimethylformamide (DMF) and carbon-tetrachloride (CCl<sub>4</sub>) were reagent grade from Merck (Darmstad, Germany). Methanol (Lichrosolv, Merck)/water purified via Milli-Q (Millipore, Bedford, MA, USA) degassed by ultra sounds (Bandelin Sonorex RK 100, Berlin, Germany) (25:75) as mobile phase. Flow rate was 1.5 ml/mn. A liquid chromatograph Gilson (Villiers-le-Bel, France) with two pumps model 302, mixing chamber, injection valve with 20 µl loop, UV detector model 116 at 316 nm with 0.01 AUFS and a reverse phase column Spherisorb S10 ODS2 250X4.6 mm (Phase Separations Ltd., Clwyd, Gt. Britan) were used.

A waring blender Moulinex (Lisbon, Portugal) with a steel blade and a 40 mesh tammy was used to sample preparation. Magnetic-mixer Selecta Agimatic N (Barcelon, Spain) regulated to 500 rpm at 60°C and a centrifuge Selecta Macrotronic regulated to 320g during 5 mn were used in extraction process.

Extract filtration was performed by glass G<sub>1</sub> filter (100 µm porosity).

### Standard solutions

Weigh accurately 50.0 mg of ronidazole (Merck Sharp and Dohme B.V., Haarlem, The Netherlands) into a 100 ml volumetric flask and solve

it in DMF. Introduce 2 ml of the previous solution into 100, 50, 25 and 20 ml volumetric flasks and complete the volume with DMF. Then get 10 ml out of each solution to a 50 ml volumetric flasks and adjust the volume with  $\text{CCl}_4$ . These solutions contain respectively 2, 4, 8 and 10  $\mu\text{g}$  of ronidazole / ml.

To prepare standard solutions of dimetridazole (Salisbury Laboratories, Inc., Charles City, IA, USA) proceed according to ronidazole.

### Procedure

Weigh 10.0 g of a previously ground and sieved sample to a 250 ml erlenmeyer, put a magnetic bar and add 20 ml of DMF and 60 ml of  $\text{CCl}_4$ . Adapt an air condensator and put in the magnetic mixer during 30 min..Then cool, filter through G<sub>1</sub> under vacuum and wash the residue with 10 ml of  $\text{CCl}_4$ . Transfer to a 100 ml volumetric flask and adjust the volume with  $\text{CCl}_4$ .

Introduce 25 ml of the filtered extract in a centrifuge tube, add 45ml of water, stirr vigourously for 2 min and centrifuge. Inject 20  $\mu\text{l}$  of the aqueous supernatant in the chromatographic system [15].

## RESULTS AND DISCUSSION

The calibration plots for the standards dimetridazole and ronidazole were obtained by plotting the mean peak area versus concentration of dimetridazole and ronidazole. The plots were linear with a mean correlation coefficient of 0.9988 for dimetridazole and 0.9998 for ronidazole.

Figure 3 shows a representative chromatogram of the 120,48 ng dimetridazole and of the 121,20 ng ronidazole standards with retention times of  $t \approx 8,5$  and  $t \approx 3,7$  minutes, respectively. A chromatogram of a feed sample with dimetridazole is shown in figure 4. We spiked feed samples with ronidazole and dimetridazole at different levels ( 2,02 to 7,07 ppm for ronidazole and 2,01 to 7,03 ppm for dimetridazole), that were subjected to the procedure described for the sample. Figure 5 shows a chromatogram of a spiked sample.

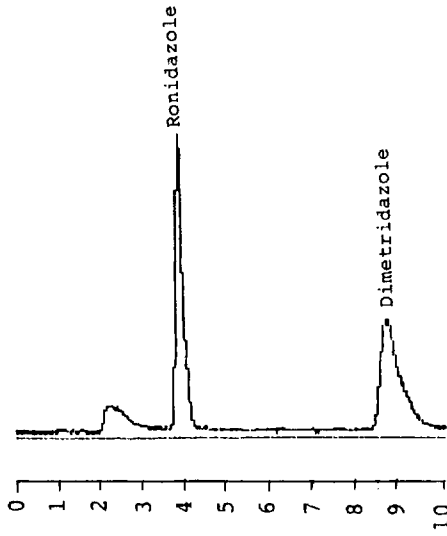


Figure 3 - Chromatogram of ronidazole and dimetridazole standards

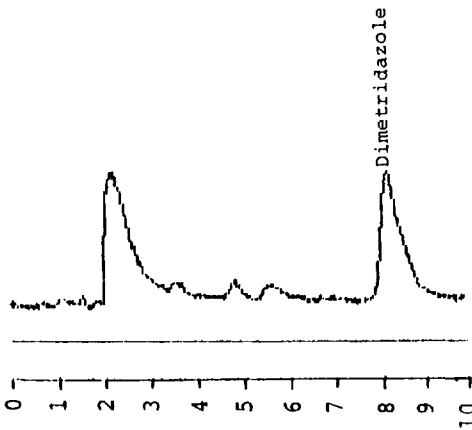


Figure 4 - Chromatogram of a sample with dimetridazole

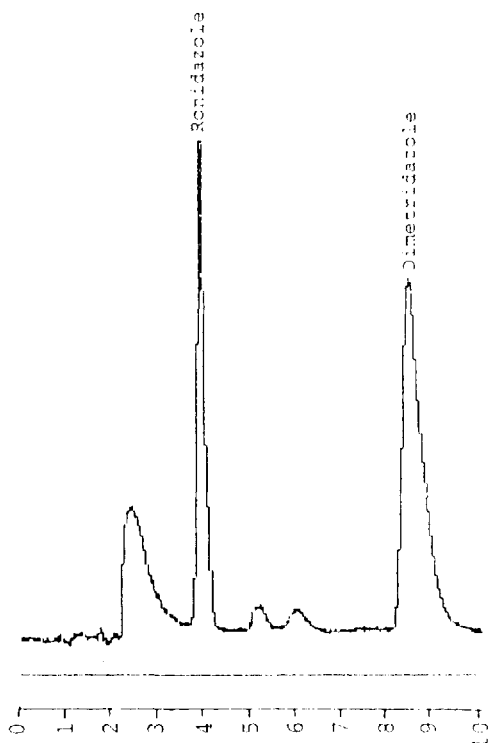


Figure 5 - Chromatogram of a sample supplemented with 2.02 ppm of ronidazole and 2.01 ppm of dimetridazole

The recoveries of ronidazole and dimetridazole from fortified samples, based on the peak areas, are given in table 1 and table 2. To ronidazole the recoveries ranged from 98,0% to 100,7% (SD 1,0 to 4,4) in samples spiked between 2,02 to 7,07 ppm levels. To dimetridazole, the recoveries ranged from 93,79% to 97,3% (SD 5,0 to 6,0) in samples spiked between 2,01 to 7,03 ppm levels.

Precision of the method was analysed by repeated determinations of carefully prepared feed samples. Intraassay coefficient variation (C.V.) of five samples on a single day ranged from 4.09% to 9.99% for

**TABLE 1**  
Recoveries of ronidazole added to feed at 2.02, 5.05, and 7.07ppm levels.

Fortification levels ppm	Recovery %
2.02	101
2.02	103
2.02	98
Average $\pm$ SD	100.7 $\pm$ 2.5
5.05	99
5.05	101
5.05	100
Average $\pm$ SD	100.0 $\pm$ 1.0
7.07	101
7.07	100
7.07	93
Average $\pm$ SD	98.0 $\pm$ 4.4

dimetridazole. For ronidazole intraassay C.V. of three samples in a single day ranged from 2.46% to 5.20% with a mean of 3.42%.

Eighty percent of the 15 analysed samples contained dimetridazole while ronidazole was only present in twenty percent of those samples, so it seems that in Portugal dimetridazole is much more used than ronidazole.

The feed samples with ronidazole present contents of this drug near of the lowest limit recommended by a portuguese law [10].

Forty percent of feed sample containing dimetridazole present very low contents of this coccidiostat ( $\pm$  20 mg/kg). The lowest contents of dimetridazole in the feed samples could be owing to manufacture



**TABLE 2**  
Recoveries of dimetridazole added to feed at 2.01, 5.02 and 7.03 ppm levels

Fortification levels ppm	Recovery %
2.01	88
2.01	100
2.01	93
Average ± SD	----- 93.7 ± 6.0 -----
5.02	92
5.02	103
5.02	97
Average ± SD	----- 97.3 ± 5.5 -----
7.07	100
7.07	95
7.07	90
Average ± SD	----- 95.0 ± 5.0 -----

process (we suppose that the use of high temperature and/or humidity during granulation process could be responsible for the destruction of the drug).

In conclusion, the proposed method describes a simple, fast and reliable approach for the simultaneous determination of dimetridazole and ronidazole in feeds.

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