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COMMUNICATION

Simultaneous Determination of Thiabendazole and Mebendazole in Tablets by High-Performance Liquid Chromatography

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ABSTRACT

Reversed-phase high performance liquid chromatography using an RP 18 column (4 × 125 mm), tetrahydrofuran–acetonitrile–0.5% formic acid (5:25:70, v/v/v) as mobile phase and UV detection at 254 nm enabled the simultaneous determination of thiabendazole (TZ) and mebendazole (MZ) in tablets. The method showed linearity over 4.0 to 40.0 μg TZ/ml and 6.0 to 60.0 μg MZ/ml. The correlation coefficient r *was .9999 for both TZ and MZ. The coefficient of variation (CV) was 0.59–0.80% for TZ and 0.49–0.67% for MZ. The average recovery was 100.54–101.17% for TZ and 100.35–101.13% for MZ. The excipients of the tablets did not interfere in the proposed method. The developed method is precise, accurate, and selective for the determination of both benzimidazoles analyzed.*

Key Words: *HPLC; Mebendazole; Thiabendazole.*

INTRODUCTION

Thiabendazole (TZ) and mebendazole (MZ) are benzimidazoles used as anthelmintics in human and veterinary therapeutics. They can be administered as an isolated drug or associated with each other and/or other drugs in the same pharmaceutical formulation. Due to structural similarity (Fig. 1), their quantitative determination when in association in a unique pharmaceutical preparation requires a specific analytical method to avoid interference.

Several analytical methods have been proposed for TZ and MZ determination in pharmaceutical preparations (1–14), but none of them concern its simultaneous determination. Therefore, the aim of this work was to develop and to validate an analytical method for the simultaneous determination of TZ and MZ in commercially available tablets.

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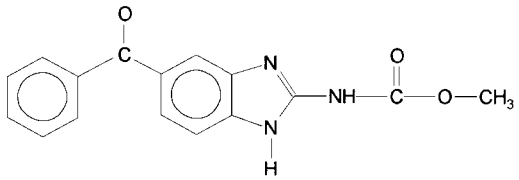
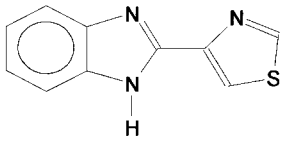


MEBENDAZOLE



THIABENDAZOLE

Figure 1. Chemical structures of benzimidazoles.

EXPERIMENTAL

Apparatus

High-performance liquid chromatographic (HPLC) separations were made on a system comprised of a CG solvent delivery pump (model 480-C) and a CG variable UV detector set at 254 nm (0.32 aufs) connected to a CG integrator (model CG-200) (Instrumentos Científicos CG Ltda., São Paulo, Brazil). The system was equipped with a manual Rheodyne 7125 injection valve fitted with a 20-µl loop. A Digimed pH meter (model T-901) and a Thornton sonicator (model T-14) were also used.

Reference Substances, Reagents, and Solutions

TZ and MZ (100.5% and 99.8%, respectively) were donated by UCI-Farma Indústria Farmacêutica Ltda. (Sao Paulo, Brazil) and were used as reference substances without further purification. All reagents and solvents were analytical grade. The solvents used in the mobile phase were HPLC grade. Distilled water was purified using a Milli-Q® system (Millipore, Milford, MA). All solutions were filtered through a hydrophilic Millipore® Durapore filtration membrane (GVWP 01300, 0.22-µm pore size).

Chromatographic Conditions

The mobile phase consisted of tetrahydrofuran–acetonitrile–0.5% formic acid (5:25:70 v/v/v). The analytical column was a LiChrospher® 100 RP-18 (5 µm) column in a LiChroCART® (125 × 4 mm) (Merck, Darmstadt, Germany). All analyses were done under isocratic conditions at a flow rate of 1.0 ml/min.

Samples

Samples 1 and 2 were commercially available tablets containing 166.0 mg of TZ and 100.0 mg of MZ per tablet, respectively. Placebos of the samples were kindly donated by their respective pharmaceutical industries.

Procedure

Standardization

Solutions containing 4.0 to 40.0 µg of TZ per milliliter and 6.0 to 60.0 µg of MZ per milliliter were prepared in 100-ml flasks by the addition of 5 ml of formic acid and 5 ml of hydrochloric acid, followed by dilution with the mobile phase. The calibration curves were constructed by plotting the peak areas against concentration (µg/ml) of TZ or MZ.

Assay

Standard Solution Preparation

For the standard solution preparation, 50.0 mg of TZ and 30.0 mg of MZ were accurately weighed and transferred to a 100-ml volumetric flask containing 5 ml of formic acid and 5 ml of hydrochloric acid. The volume was completed with the mobile phase. An aliquot of 2.0 ml was transferred to a 50-ml volumetric flask, and the volume was made up with the mobile phase. This solution contained 20.0 µg of TZ per milliliter and 12.0 µg of MZ per milliliter.

Sample Preparation

An accurately weighed amount of the powdered tablets equivalent to 50.0 mg of TZ and 30.0 mg of MZ was transferred to a 100-ml volumetric flask containing 5 ml of formic acid and 5 ml of hydrochloric acid. After the addition of 20 ml of mobile phase, the mixture was sonicated for 12 min, and the volume was completed with mobile phase. The solution was filtered, and the first 10 ml were rejected. A 2.0-ml aliquot was transferred to a 50-ml volumetric flask, and the volume was made up with the mobile phase. The resulting solution was passed through a membrane filter (0.22-µm pore size), and 20 µl of each sample solution (10 injections of each sample) and 20 µl of standard solution in the same concentration (3 injections) were injected in the chromatograph.

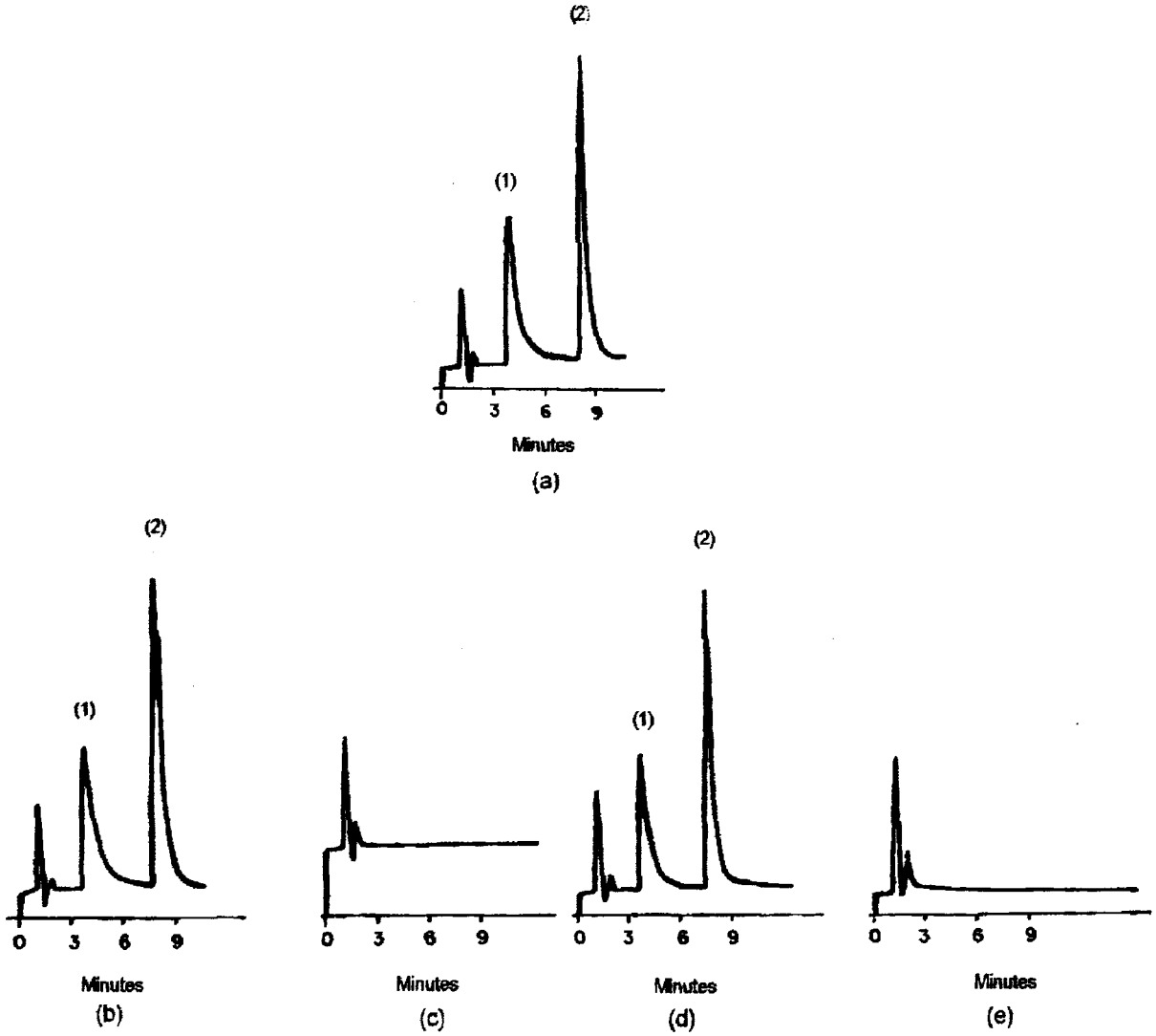


Figure 2. Chromatograms of thiabendazole and mebendazole contained in tablets. Conditions were as follows: LiChrospher 100 RP-18 (5 μm) in LiChroCART (125 × 4 mm) column; mobile phase of tetrahydrofuran–acetonitrile–0.5% formic acid (5:25:70 v/v/v); UV detection at 254 nm; room temperature; 20 μl loop; and 1.0 ml/min flow rate. (a) Standards: (1) thiabendazole (20.0 μg/ml), (2) mebendazole (12.0 μg/ml); (b) sample 1; (c) placebo of sample 1; (d) sample 2; (e) placebo of sample 2.

RESULTS AND DISCUSSION

The proposed HPLC method enabled the simultaneous quantitative determination of TZ and MZ in an associated form. Samples were prepared by dissolving the drugs in formic acid and hydrochloric acid, followed by dilution with the mobile phase and filtering to eliminate the insoluble excipients. The excipients seemed to produce no interference in the proposed method (Fig. 2).

A wavelength of 254 nm was selected to facilitate simultaneous determination of both active substances in the tablets.

The calibration curves were obtained in a concentration range from 4.0 to 40.0 μg of TZ per milliliter and from 6.0 to 60.0 μg of MZ per milliliter. The regression curves were calculated by the least-squares method. The correlation coefficients were 0.9999 for both TZ and MZ, and the relative standard errors of estimates were 1.64% for TZ and 1.03% for MZ (Table 1).

Table 2 shows the results obtained in the determination of TZ and MZ in commercially available samples. The coefficients of variation and the confidence limits (p = 95%) of the results are also presented in Table 2.

The recovery tests were performed according to the

Table 1

Statistical Data Obtained in Standardization of the High-Performance Liquid Chromatographic Method for Determination of Thiabendazole and Mebendazole in Tablets

Parameter	Thiabendazole	Mebendazole
Linearity (μg/ml)	4.0–40.0	6.0–60.0
Slope (*b*)	6385.39	17,422.23
Intercept (*a*)	1974.20	4518.20
Correlation coefficient (*r*)	.9999	.9999
Relative standard error of estimation (%)	1.64	1.03

Table 2

Results Obtained in the Determination of Thiabendazole (TZ) and Mebendazole (MZ) in Commercial Samples by Proposed Method

	Declared Amount (mg/tablet)		Found Amount[a] (%) ± Confidence Limit		Coefficient of Variation (%)	
Sample	TZ	MZ	TZ	MZ	TZ	MZ
1	166.00	100.00	98.42 ± 0.41	102.70 ± 0.49	0.59	0.67
2	166.00	100.00	99.55 ± 0.57	101.68 ± 0.36	0.80	0.49

[a] Average of 10 determinations.

Table 3

Recovery of Standard Thiabendazole Added to Commercially Available Tablets (Samples 1 and 2) Using High-Performance Liquid Chromatography

Sample	Added (μg/ml)	Found[a] (μg/ml)	Recovery (%)
	5.00	5.02	100.40
1	10.00	10.05	100.50
	15.00	15.11	100.73
	5.00	5.07	101.40
2	10.00	10.17	101.70
	15.00	15.06	100.40

[a] Average of 2 determinations.

Table 4

Recovery of Standard Mebendazole Added to Commercially Available Tablets (Samples 1 and 2) Using High-Performance Liquid Chromatography

Sample	Added (μg/ml)	Found[a] (μg/ml)	Recovery (%)
	3.00	2.93	97.67
1	6.00	6.21	103.50
	9.00	9.20	102.22
	3.00	3.02	100.67
2	6.00	6.03	100.50
	9.00	8.99	99.89

[a] Average of 2 determinations.

Association of Official Analytical Chemists International (AOAC International) method (15). TZ and MZ were added to sample solutions. The recovery data presented in Tables 3 and 4 confirm the accuracy of the method.

The proposed method is rapid, precise, accurate, and efficient and allows the simultaneous quantitative determination of TZ and MZ in a pharmaceutical preparation. The proposed method can also be used as an alternative to the existing methods for single determination of TZ or MZ in pharmaceutical formulations.

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