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Development of a Discriminative Dissolution Method for Mebendazole Suspensions

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ABSTRACT

Introduction: Studying the dissolution profile is a suitable method for the comparison of novel or special dosage forms, including oral and parenteral suspensions, soft gel capsules, and transdermal patches. Mebendazole is a broad-spectrum benzimidazole approved by United States Food and Drug Administration to treat different parasitic diseases. Despite its wide use, there is no compendial dissolution requirement for oral suspensions. The objective of this study was to develop a discriminatory in vitro release test for mebendazole suspensions. **Methods:** United States Pharmacopeia (USP) apparatus 2 (paddle) and 4 (flow-through cell) were selected, and 0.1 N hydrochloric acid with 1% sodium lauryl sulfate was used as the dissolution medium. In apparatus 2, a 50-rpm agitation rate and sample insertion mode in the dissolution vessel were evaluated. In apparatus 4, flow rate, bed size, and open and closed configurations were assessed. Dissolution profiles of three commercial products were analyzed. **Results:** Dissolution studies using USP apparatus 2 showed that all products complied with very rapid dissolution criteria, and the method was not able to discriminate between products. For apparatus 4, a flow rate of 16 mL/min was selected. No differences in dissolution behavior for the reference product were found between open and closed-loop configuration. Statistical differences in dissolution profiles were found among products using the open-loop configuration. **Conclusion:** USP apparatus 4 with the open-loop configuration had more discriminatory power than apparatus 2 in assessing the dissolution release from oral mebendazole suspension products. The developed method could be suitable for quality control and dissolution profile comparison of mebendazole commercial formulations.

KEYWORDS: Mebendazole suspension, dissolution, USP apparatus 4, USP apparatus 2

INTRODUCTION

Mebendazole (MBZ) is an antiparasitic drug that has shown efficacy against a broad spectrum of intestinal helminths. MBZ is included in the World Health Organization's Model List of Essential Medicines (*1*). In Mexico, MBZ is approved and frequently prescribed for the treatment of *T. trichiura, A. lumbricoides, E. vermicularis, A. duodenale, N. americanus, T. solium, and T. saginata* (*2*). Recently MBZ has been repositioned as a promising anti-cancer drug in various types of cancer like brain, lung, breast, and colon, acting through different molecular mechanisms including tubulin disruption and VEGFR2-mediated anti-angiogenesis (*3*). MBZ is a highly lipophilic (log P = 3.09), amphiprotic molecule (pka1 = 3.43, pka2 = 9.6) (*4*). One of the main problems related to MBZ's poor bioavailability is its extremely low solubility (*5*). The drug has been classified as a class 2 drug in the Biopharmaceutics Classification System (BCS) (low solubility and high permeability) (*6*). To date, there are different MBZ dosage forms available in the market, including 100–500-mg tablets, 100–500-mg chewable tablets, and in some countries a 20 mg/mL suspension is available. Although the United States Pharmacopeia (USP) as well as Argentinian and Mexican Pharmacopoeias include a dissolution test in the tablet monograph, no compendial requirement is available for the release characteristics of this drug in suspension (*7–9*). Suspensions are dispersed systems

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in which drug insoluble solid particles are distributed uniformly in a liquid medium. Various factors are related with the dissolution rate of dispersed systems, including physicochemical properties (particle size), formulation characteristics, and viscosity (*10*).

Considering that the dissolution test is an important tool for quality control of pharmaceutical formulations, the main objective of the present study was to develop a discriminative dissolution test for MBZ suspension products.

METHODS

Chemicals

MBZ analytical standard was obtained from Supelco (USA). Sodium lauryl sulfate (SLS) 95% was obtained from Comercializadora Garnica (Mexico), and formic acid was purchased from Sigma Aldrich (USA). Hydrochloric acid (HCl), methanol (high-performance liquid chromatography [HPLC] grade) and acetonitrile (HPLC grade) were purchased from J.T. Baker (USA). Water was obtained from a water purification system (Milli-Q, Merck Millipore, Germany).

Drug Products

For the dissolution studies, three different 20-mg/mL oral suspensions of MBZ were selected. One was the reference product Vermox (A) and the other two were generic products (B and C).

MBZ products were obtained directly from local pharmacies in Mexico City, Mexico. All products were within the expiration date. Density was evaluated according to the *Mexican Pharmacopoeia* (*9*).

Solubility

MBZ solubility was assessed in 0.1 N HCl alone and in HCl with 0.5% and 1% SLS using the shake flask method. MBZ was added in excess to 5 mL of each media (n = 3). The tubes were placed on a plate shaker and maintained at 25 ± 0.5 °C with constant stirring for 72 h. Tubes were centrifuged at 4000 rpm for 15 min, then an aliquot of the supernatant was obtained and analyzed spectrophotometrically at 287 nm.

Dissolution Studies with USP Apparatus 2

To select the best conditions for the insertion of the sample in the dissolution vessel, three conditions were evaluated using the reference product: at the bottom of the vessel, in the middle, and at the surface of the dissolution media. After mechanical agitation, an amount of suspension containing approximately 100 mg of MBZ was introduced in the vessels, which was determined by weighing a syringe before and after the sample introduction and according to the density of each product. Then, a 5-mL sample was taken at 30 min, filtered through a 0.45-µm Durapore filter (Millipore Sigma, USA), diluted with the dissolution media, and analyzed spectrophotometrically. The amount dissolved was determined using a spectrophotometrically validated method at 287 nm (UV-VIS spectrophotometer, UV-1900, Shimadzu, Japan). Validation of the method was performed according to the Mexican guidance (*11*). The method was linear from 1–13 µg/mL, with intra- and interday coefficients of variation less than 2%.

Dissolution studies were carried out with the reference and two generic products (n = 6) using USP apparatus 2 (paddle) (708-DS, Agilent, USA). The dissolution media was 900 mL of 0.1 N HCl containing 1.0% of SLS at 37 ± 0.5 °C. The amount of suspension was introduced as described above, then the paddle rotation speed was set at 50 rpm. Samples (5 mL) were withdrawn without medium replacement at 10, 20, 30, 60, and 90 min, and filtered through a 0.45-µm Durapore filter. Then, samples were diluted and assayed at 287 nm.

Dissolution Studies with USP Apparatus 4

Additional dissolution studies were performed using USP apparatus 4 (flow-through cell) (CE 7 Smart Sotax with Win SOTAX Plus, Switzerland,) and 22.6-mm diameter cells. A 5-mm diameter ruby pearl was placed at the bottom of the cell, and 1-mm diameter glass beads were used.

To select the best conditions for the dissolution studies, two variables were evaluated: flow rate and arrangement of the glass beads. All tests were performed with the reference drug using a sandwich-type arrangement with 3 g of glass beads each (top and bottom of the cell) and a 20-mg suspension. The flow rate conditions evaluated were 8 and 16 mL/min. In both conditions, samples were withdrawn at 30, 60, and 90 min, and the percentage of drug dissolved was determined.

It has been shown that dissolution behavior can be influenced by the arrangement of glass beads in the cells, owing to differences in hydrodynamics. Thus, six conditions were tested: 1) 5 g of glass beads homogenized with a 20-mg suspension; 2) 3 g of glass beads and a 20-mg suspension; 3) sandwich arrangement with 10 g of glass beads on bottom, 20-mg suspension in the middle, and 5 g of glass beads on top; 4) sandwich arrangement with 3 g of glass beads on bottom, 20-mg suspension in the middle, and 3 g of glass beads on top; 5) sandwich arrangement with 2 g of glass beads on bottom, 20-mg suspension in the middle, and 2 g of glass beads on top; and 6) 20-mg suspension without glass beads. For all of these conditions, samples were obtained at 60 min using

a flow rate of 16 mL/min and the percentage of drug dissolved was determined.

Once the dissolution conditions were selected, the dissolution profiles of the reference product were evaluated in both closed- and open-loop configurations using a flow rate of 16 mL/min.

According to the density of each suspension, an amount equivalent to 20-mg MBZ was placed in each cell (n = 12). The dissolution media was 0.1 N HCl with 1% SLS at 37 ± 0.5 °C, using sandwich arrangement with 3-g glass beads, 20-mg suspension, and 3-g glass beads, and a flow rate of 16 mL/min. Samples were withdrawn at 10, 20, 30, 45, 60, 90, and 120 min, and filtered through a 0.45-µm Durapore filter. Then, samples were diluted with the dissolution media and analyzed spectrophotometrically at 287 nm.

Statistical Analysis

To determine the kinetics of drug release, the Microsoft Excel add-in, DDSolver was used. Data were fitted to the first order and Weibull kinetic release models. The determination coefficient and the Akaike criterion (AIC) were used to select the optimal model. For dissolution profile comparison in apparatus 4, similarity factor analysis (f_2 test and f_2 bootstrap) was performed.

RESULTS AND DISCUSSION

Considering the importance of MBZ in the treatment of helminth diseases, we evaluated different procedures to determine the release characteristics of MBZ suspension products from the Mexican market. The reference product A was white, and generic products B and C were yellow and pink, respectively. The relative density values were 1.12, 1.13, and 1.09 g/mL), respectively.

The solubility results showed that in 0.1 N HCl, MBZ solubility was low (79.76 µg/mL); however, when 0.5% SLS and 1% SLS were added, solubility increased to 209.5 and 488.64 µg/mL, respectively. Based on these results, 0.1 N HCl containing 1.0% SLS was selected as dissolution media to maintain sink conditions in the dissolution studies.

USP Apparatus 2

Using the paddle apparatus, the mean percentage of drug dissolved (± relative SD [RSD]) at 30 minutes when the sample was placed at the surface, in the middle, and at the bottom of the dissolution vessel were 90.51% (1.18), 91.63% (10.64), and 91.19% (2.61) respectively. Considering the similarity of these values, the introduction of the sample at the surface of the vessel was selected, due to the ease of the sample positioning and the low variability.

Figure 1 shows the dissolution profiles of MBZ products in USP apparatus 2 at 50 rpm with 0.1-N HCl and 1% SLS as the dissolution medium. All products complied with the very rapid dissolution criteria (more than 85% of dose dissolved in 15 min); therefore, f_2 was not calculated. However, this method was not able to discriminate between products.

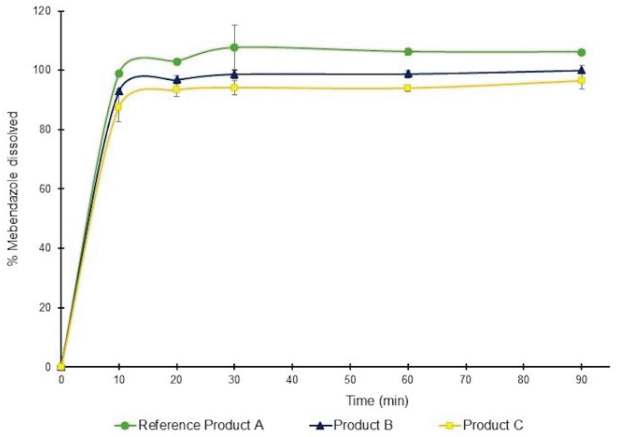

Figure 1. Cumulative dissolution profiles of mebendazole suspensions using apparatus 2, dissolution media 900 mL of 0.1 N HCl with 1.0% of sodium lauryl sulfate at 37 ± 0.5 °C and 50 rpm. Data are presented as mean ± relative SD (n = 6).

USP Apparatus 4

The percentages of MBZ dissolved using flow rates of 8 and 16 mL/min in USP apparatus 4 are shown in Table 1. No direct relationship was observed between flow rate and the percentage dissolved. The flow rate of 16 mL/min was selected because the RSD was lower at the different sampling times.

Table 1. Percentage of Mebendazole (MBZ) Dissolved From Suspension Using Different Flow Rates in USP Apparatus 4

Time (min)	Flow Rate: 8 mL/min	Flow Rate: 16 mL/min
	MBZ Dissolved (%), mean (RSD)	MBZ Dissolved (%), mean (RSD)
30	45.9 (47.2)	55.2 (0.60)
60	64.9 (33.8)	73.8 (1.12)
90	76.9 (24.1)	82.0 (1.61)

USP: United States Pharmacopeia; RSD: relative standard deviation.

Table 2 shows the percentage of MBZ dissolved at 60 min in apparatus 4 with the different arrangements of the glass beads. The lowest percentage and most variability were obtained without glass beads, which could be associated with the nonhomogeneous flow of the dissolution medium. In previous studies, it has been reported that when the cell is operated without glass beads, then the flow is turbulent, whereas a laminar flow is obtained when glass beads are used (*12*). The

arrangements 1 and 2, in which the suspension was mixed with the beads presented a moderate release of MBZ. This arrangement has been recommended for the evaluation of powders; however, it was not suitable for MBZ suspension (*13*). Considering that the sandwich-type arrangement has been recommended for evaluation of drug release of suspensions, three different arrangements were evaluated (*14*). The highest percentages of dissolved MBZ were obtained with arrangements 4 and 5. The least variability was observed in arrangement 4, which could be related to a more homogeneous laminar flow associated with a pressure drop provoked by the beads; however, the addition of beads at the bottom and top of the cell (arrangement 3) yielded less drug release, which could be related to reduced diffusion of dissolution media through the beads, thereby reducing contact with the drug.

Figure 2 shows that the dissolution profiles of the reference product were similar when the open- and closed-loop configurations were used (f_2 = 66.7). The open loop was selected to obtain the non-cumulative dissolution profiles.

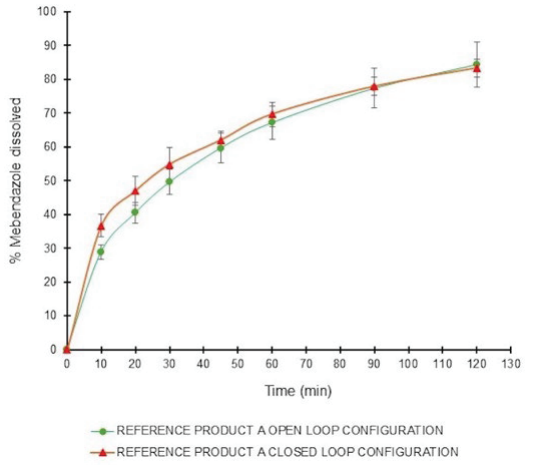

Figure 2. Dissolution profiles for mebendazole reference product using a flow-through cell apparatus at 16 mL/min with closed- and open-loop configuration.

Figure 3A shows the dissolution profiles of the products under study in the flow-through cell apparatus. Wide variability was obtained with product B, so the bootstrap f_2 method was used to compare the dissolution profiles of the products B and C with the reference. Results were 36.35 and 45.81 for products B and C, respectively. These data demonstrate the discriminatory power of the developed method. Figure 3B shows the non-cumulative release of MBZ. The slowest release rate was obtained for product B.

Table 3 shows the kinetic modeling parameters for MBZ release using apparatus 4, with model dependent and independent methods. The model that provided the best fit was the Weibull model, because it had the highest the correlation coefficient and the lowest AIC value. The variables of the Weibull model are α, which defines the time scale of the dissolution process; β which represents a shape factor of the dissolution curve (β = 1 exponential shape, β < 1 parabolic shape, β > 1 sigmoidal shape), and Td, which represents the time of the release of the 63.2% of the drug. Results showed that product B presented the largest difference in Td, mean dissolution time, and dissolution efficiency.

This is the first dissolution study of MBZ oral suspension. Our results showed that when the USP apparatus 2 was used, products were rapidly dispersed in the vessel and the dissolution was faster than in USP apparatus 4 using the same dissolution medium. Differences in the dissolution profiles could be associated with the differences in the hydrodynamic conditions in both systems; in the flow-through cell apparatus, the operation in open-loop configuration allows a laminar flow, which could be more representative of the laminar flow found in the gastrointestinal tract (*15*, *16*). Also, apparatus 4 has been shown to be a more discriminating method for poorly soluble compounds, and the current

Table 2. Percentage of Mebendazole (MBZ) Dissolved From Suspension After 60 min Using Different Arrangements of Glass Beads in USP Apparatus 4

Arrangements	MBZ Dissolved (%), mean	RSD (%)
1. 5 g of glass beads and 20 mg of suspension	54.5	6.54
2. 3 g of glass beads and 20 mg of suspension	56.57	0.41
3. Sandwich-type arrangement with 10 g of glass beads on bottom, 20 mg of suspension, and 5 g of glass beads on top	24.22	5.41
4. Sandwich-type arrangement with 3 g of glass beads on bottom, 20 mg of suspension, and 3 g of glass beads on top	70.38	2.98
5. Sandwich-type arrangement with 2 g of glass beads on bottom, 20 mg of suspension, and 2 g of glass beads on top	66.07	7.24
6. Without glass beads	20.23	16.29

USP: United States Pharmacopeia; RSD: relative standard deviation.

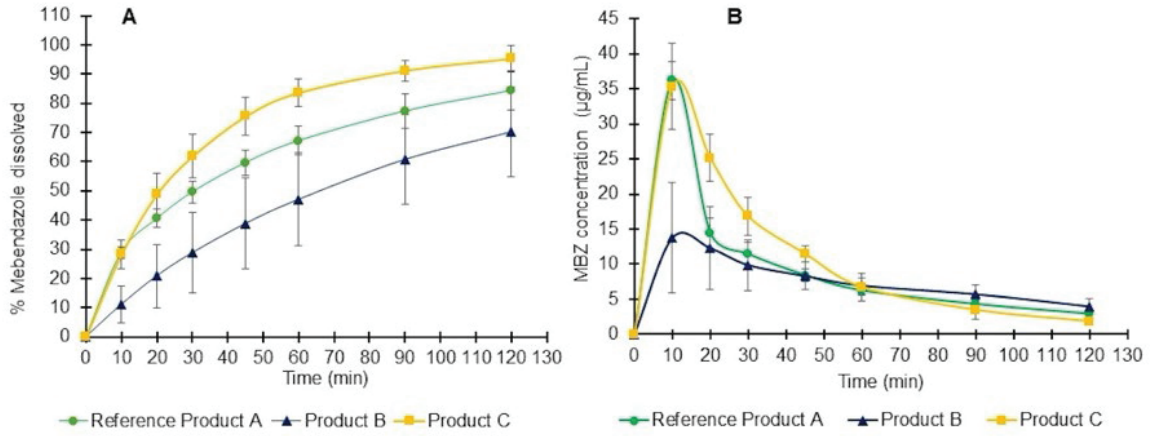

*Figure 3. (**A**) Cumulative dissolution profiles of mebendazole (MBZ) suspensions using USP apparatus 4 in an open-loop configuration and a flow rate of 16 mL/min. (**B**) Non-cumulative dissolution profiles. Data are mean ± SD (n = 12).*

Table 3. Dissolution Data Modeling for Mebendazole (MBZ) Suspension Products Using DDSolver in USP Apparatus 4

Model	Parameter	Product		
		A (Reference)	B (Generic)	C (Generic)
Weibull	α	14.48	69.62	27.94
	β	0.632	0.936	0.993
	Fmax	111.4	97.7	95.4
	Td	68.7	93.0	28.6
	R^2	0.997	0.997	0.990
	AIC	11.97	14.26	24.11
First order	k1	0.032	0.0129	0.0351
	Fmax	82.74	88.79	95.29
	R^2	0.965	0.998	0.998
	AIC	34.27	14.66	14.88
Independent	MDT	33.33	47.02	27.21
	DE	0.61	0.43	0.73
	Kd	0.03	0.021	0.037

USP: United States Pharmacopeia; α: scale parameter; β: shape parameter; Fmax: maximum percentage of drug dissolved; Td: time to release 63.2% of the drug; AIC: Akaike information criterion; k1: first order dissolution constant; MDT: mean dissolution time; DE: dissolution efficiency; Kd: dissolution constant calculated as 1/MDT.

study results are in agreement with those reported for low solubility compounds. For example, Medina et al. evaluated the dissolution profile of carbamazepine (BCS class 2) immediate-release tablets and found that the dissolution rate using the flow-through cell apparatus was slower than that found with the paddle apparatus (*17*). The authors discussed that this fact could be related to the hydrodynamics of the system, where no agitation mechanism exists, and the dosage form and the drug particles were continuously exposed to a uniform laminar flow, causing a different dissolution pattern (*17*). In another study, Polski et al. found that with the open-loop flow-through cell system, the release of papaverine hydrochloride (BCS class 2) was slower than in the basket or paddle apparatus (*18*). Furthermore, Solis-Cruz et al. evaluated the dissolution profile of metoprolol tartrate immediate-release tablets (BCS class 1) and reported that the apparatus 4 method was more reproducible and efficient because the workflows were constant, unlike other dissolution systems (*19*). Considering these studies, USP apparatus 4 could be a suitable option for oral suspensions with low solubility drugs such as MBZ.

CONCLUSIONS

Although USP, Mexican, and Argentinian Pharmacopeias include a monograph for MBZ oral suspension, there is no dissolution test available for this pharmaceutical dosage form. The results of the present study showed that the flow-through cell apparatus with open-loop configuration using 0.1 N HCl with 1% SLS as dissolution media at 37 ± 0.5 °C and a sandwich arrangement at 16 mL/min had more discriminatory power than the paddle apparatus.

This method could be suitable for quality control and dissolution profile comparisons of MBZ commercial formulations in suspension.

DISCLOSURES

The authors received no financial support for this work and have no conflicting interests.

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