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Research paper

Quality evaluation of generic drugs by dissolution test: changing the USP dissolution medium to distinguish between active and non-active mebendazole polymorphs

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Abstract

Mebendazole is practically insoluble in water and studies of its polymorphism has led to the identification and characterization of three polymorphic forms (A, B, C) displaying solubility and therapeutic differences that show that polymorph C is therapeutically favored. The objective of this study was to adjust the USP dissolution test for mebendazole so that it was able to distinguish between the dissolution properties of three mebendazole polymorphs. This would provide generic manufacturers with one more test to ensure that the therapeutically active polymorph C is used. The results obtained in this study show that the USP dissolution test conditions were not able to distinguish between the dissolution properties of completely dispersed mebendazole polymorphs with comparable particle sizes. When sodium lauryl sulfate was removed from the dissolution medium, the percentage dissolved versus time profiles changed so that polymorph C dissolved faster (70% within 120 min) compared to polymorph B (37% within 120 min) and polymorph A (20% within 120 min).

Keywords: Mebendazole; Dissolution; Polymorph; Pharmacopeial; Surfactant

1. Introduction

In a recent letter published in the *South African Medical Journal*, Evans et al. [1] asked for the establishment of a procedure to ensure that all batches of imported mebendazole raw material and tablets contain the molecular, or rather, crystal polymorph C which has been demonstrated to be, the most efficacious form [2–4]. Mebendazole is a broad-spectrum anthelmintic drug producing high cure rates in infestations by *Ascaris*, threadworms, hookworms and whipworms [5]. The drug is practically insoluble in water and studies of its polymorphism has led to the identification and characterization of three polymorphic forms (A, B, C) displaying solubility and therapeutic differences [2,3,6]. The polymorphs differ with respect to their spectral and thermal properties [2,5]. In the DSC thermograms of the three polymorphs, a common endotherm at 235 °C is observed which represents the thermal decomposition of the drug. The thermograms of forms B and show additional endotherms at 210 and 170 °C, respectively. The solubility of the three polymorphs in 0.03 M hydrochloric acid is in the order A < C < B [4]. Solubility studies and clinical trials have shown that polymorph C is therapeutically favored [3,4,7].

In one therapeutic trial the use of mebendazole, 300 mg polymorph A, 300 mg polymorph C and 500 mg polymorph C, in the treatment of hookworm and Trichuris infections was carried out at primary schools in Southern Thailand [7]. A total of 958 children were randomly allocated in seven treatment groups including the placebo control and the standard dose control (100 mg polymorph C b.i.d. for 3 days). The egg reduction rates and the cure rates of 300 and 500 mg polymorph C were similar, while the efficacy of single dose 300 mg polymorph A was not different from that of the placebo control in both infections. In a further attempt to test the biological activity of three polymorphic forms of mebendazole, forms A, B and C, both the LD_{50} in mice after oral and intraperitoneal administration and the anthelmintic

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effect of these forms on the enteral and parenteral phases of the nematode *Trichinella spiralis* were studied. They observed that the polymorphic form A was the least toxic and effective against *T. spiralis*.

In developing countries, pharmaceutical manufacturers and regulatory agencies usually rely on pharmacopeial tests to ensure the quality of pharmaceuticals. In the USP [8], BP [9] and EP [10] there are monographs describing several chemical tests for mebendazole raw material, tablets, and suspensions. However, none of these tests, except perhaps the IR identification test and possibly the dissolution test described in the USP, can be used to distinguish between crystal polymorphs [4,6]. The objective of this study was to adjust the USP dissolution test for mebendazole so that it was able to distinguish between the dissolution properties of mebendazole polymorphs. This would provide generic manufacturers with one more test to ensure that the therapeutically active polymorph C is used.

2. Materials and methods

The mebendazole polymorphs were identified amongst raw material samples obtained from a number of manufacturers and were prepared by recrystallization [2,4]. Form A was recrystallized from glacial acetic acid, Form B from chloroform and form C from methanol. The purity of the powders was between 99 and 101% as determined using the methods described in the USP [8]. Both infrared (IR) spectra and X-ray powder diffraction (XRPD) were used to characterize the three mebendazole polymorphs. IR spectra were recorded on a Nexus 470 spectrophotometer (Nicolet Instrument Corp., Madison, WI, USA) over a range of 4000–400 cm^{-1} with the Avatar Diffuse Reflectance smart accessory. Samples weighing approximately 2 mg were mixed with 200 mg of KBr (Merck, Darmstadt, Germany) by means of an agate mortar and pestle, and placed in sample cups for convenient, fast sampling. The XRPD profiles were obtained at room temperature with a Philips PM9901/0 diffractometer (Philips, Netherlands). The measurement conditions were target, Cu; filter, Fe; voltage, 40 kV; current, 20 mA; slit, 0.2 nm; scanning speed, 2°/min. Approximately 200 mg of sample was loaded into an aluminum sample holder and care taken not to introduce a preferential orientation of the crystals. Particle size distributions in suspension of all samples were measured with a Galai-Cis-1 particle size analyzer (Galai, Israel). This instrument used dual discipline analysis integrating laser diffraction and image analysis for particle sizing. Samples, 10 mg, suspended in a suitable dispersing solution (water) were placed in small cuvettes and fitted into the analyzer. A small magnetic stirrer inside the cuvette prevented sedimentation of the particles during the measurement. The acquired data were used to compute means, medians and standard deviations based on the total particle population.

Powder dissolution studies of forms A, B and C were performed using Method 2, paddle, of the USP 25 [8]. The paddles were rotated as 75 rpm and samples were withdrawn from the dissolution medium at 7.5, 15, 22.5, 30, 45, 60, 90 and 120 min. The powder sample, 50 mg, was rinsed from the glass weighing boat into a 10 ml test tube with exactly 2 ml of dissolution medium. Glass beads, 50 mg, with a mean size of 0.1 mm, were added to the suspension and the mixture was agitated for 20 s using a vortex mixer. The contents of the test tube were then transferred into the dissolution medium. The dissolution media used were 900 ml 0.1 M hydrochloric acid and 900 ml 0.1 M hydrochloric acid containing 1% sodium lauryl sulfate, this is the medium prescribed by the USP. The concentration of dissolved powder was calculated from the UV absorbance obtained at 254 nm. Results are the mean of 12 individual dissolution tests. When a powder did not comply with the USP criteria the dissolution test was repeated in accordance to USP specifications.

To determine the solubility in water, 12-ml vials containing 0.1 M HCl and excess amounts of each crystal form were rotated (100 rpm) in a water bath kept at 30 ± 0.1 °C. After 48 h, equilibrium was reached and aliquots of the solution were withdrawn from the vials and filtered through a 0.25-μm filter. The solutions were suitably diluted with methanol and assayed with a spectrophotometer at 254 nm. Results are the mean of five determinations.

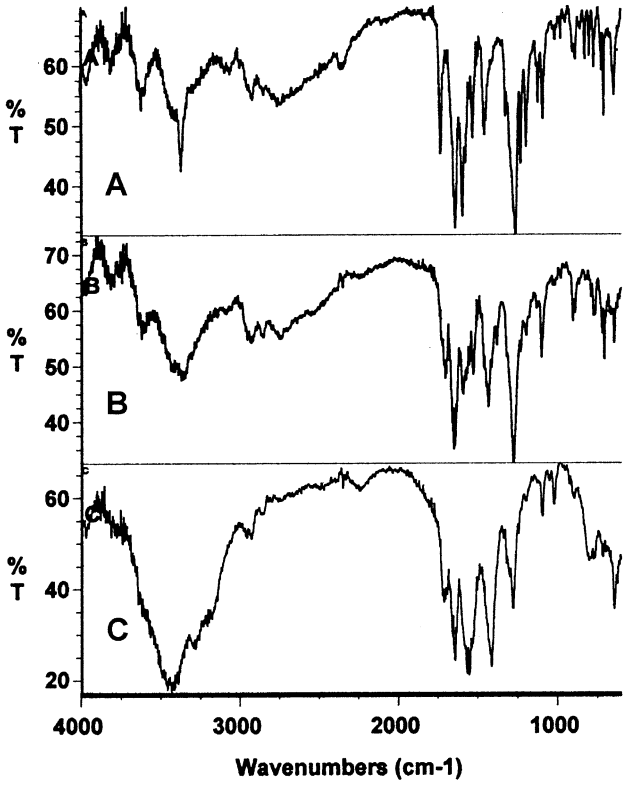

Fig. 1. FTIR spectra of the three mebendazole polymorphs.

Table 1
Characteristic spectral and physicochemical properties of mebendazole polymorphs used in this study

Form	IR (cm^{-1})		XRPD		Particle size (μm)	Solubility in 0.1 M HCl (mg ml^{-1})[a]
	–NH	–C=O	d (Å)	I/I_1 (%)		
A	3370	1730	11.52	100	5.36 ± 1.54	0.02 ± 0.005
			5.13	70		
			3.84	47		
			3.11	43		
B	3340	1700	4.65	100	6.18 ± 1.72	0.07 ± 0.004
			9.36	83		
			3.62	67		
			4.11	63		
C	3410	1720	4.48	100	5.35 ± 1.02	0.04 ± 0.003
			3.32	73		
			17.91	72		
			3.59	54		

[a] Costa et al. [4].

3. Results

Infrared spectroscopy (Fig. 1) has emerged as the preferred method to identify the polymorphic forms of mebendazole [5,6]. In Table 1 some physicochemical properties and the main IR signals and XRPD intensities for the powders used in this study are listed. XRPD patterns were similar to those reported by Costa et al. [4]. Together with the characteristic IR-signals and data as provided in Table 1, confirmed that the three powders tested were the three polymorphs of mebendazole. The solubility of the powders in 0.03 M HCl are reported to be in the order B > C > A [4]. Similar results listed in Table 1 were obtained in this study when the solubility was measured in 0.1 M HCl at 30 °C. The mean volume particle sizes of the powders (Table 1) were approximately the same. Differences in dissolution should therefore not be due to differences in particle size. All three powders were cohesive and did not flow easily. Light microscopy (Fig. 2) revealed differences in the powder properties of the three polymorphs. Form B was a looser, freer flowing powder while the particles of form A and C were more aggregated. To eliminate the effect of particle aggregation on dissolution rate the powders were dispersed with the aid of small glass beads before it was added to the dissolution medium.

The percentage dissolved versus time for the three polymorphs, as dispersed powders with particle sizes below 10 μm, are shown in Fig. 3. According to the USP not less than 75% (Q) of mebendazole must be dissolved within 120 min. The results in Figs. 3 and 4 show that these test conditions were not able to distinguish between the solubility properties of mebendazole polymorphs. More than 75% of the polymorphs dissolved in 120 min in the USP medium, all within the USP tolerance. Form C = 100% > Form A = 98% > Form B = 94%. In 0.1 M HCl the dissolution rates were significantly lower and there were clear differences in the dissolution properties of the three polymorphs. The percentage dissolved in 120 min (Q) were in the order Form C = 70% > Form B = 37% > Form A = 20%. Compared to the USP dissol-

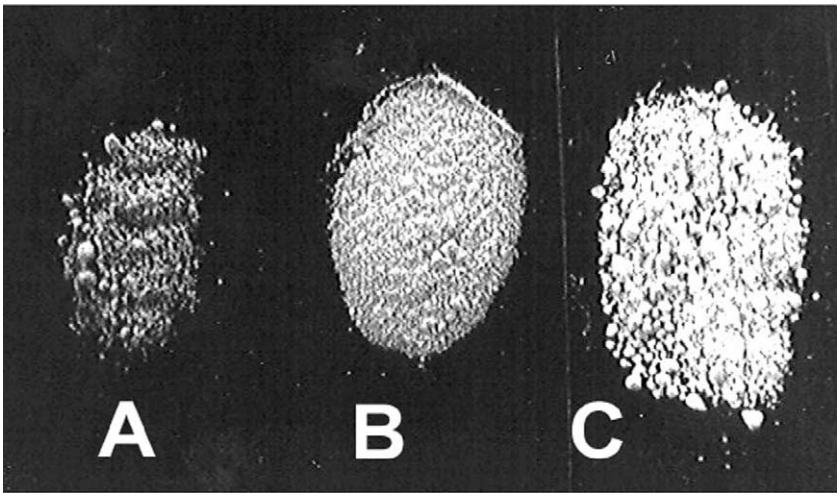

Fig. 2. Light microscope photo of the three mebendazole polymorphs.

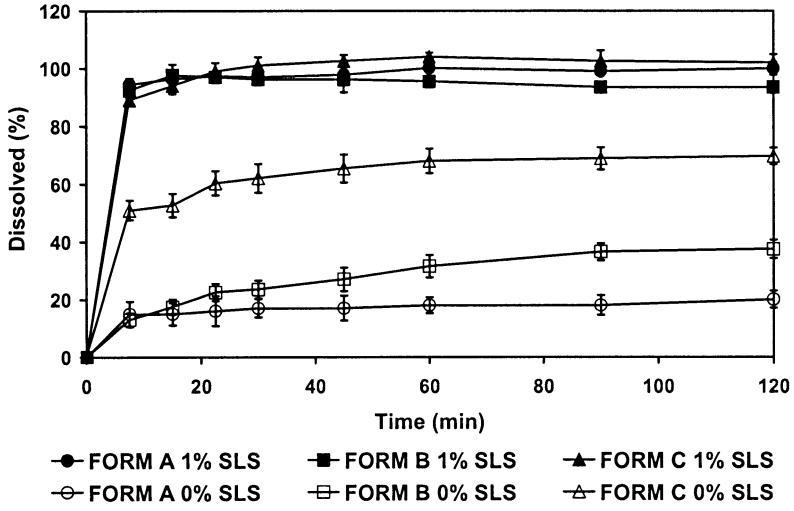

Fig. 3. Powder dissolution profiles of mebendazole polymorphs in 0.1 M HCl (open symbols) and 0.1 M HCl containing 1% sodium lauryl sulfate (closed symbols).

ution medium in 0.1 M HCl none of the powders passed the requirement of 75% dissolved in 120 min.

4. Discussion

According to the USP dissolution test for mebendazole, not less than 75% (Q) of the labeled amount of the drug must dissolve in 120 min from six individual tablets, in 900 ml of a 0.1 M hydrochloric acid solution containing 1% sodium lauryl sulfate, a surface active agent. The results obtained in this study show that these test conditions were not able to distinguish between the differences in the dissolution properties of completely dispersed mebendazole polymorphs with comparable particle sizes. Solubility studies in 0.1 M HCl have shown the solubility of mebendazole to be very low (Table 1) and in the order A < C < B [4]. Since more than 75% of the polymorphs dissolved in 120 min, all within the USP tolerance, the dissolution properties of the powder are equal in the USP medium. Under these conditions, increased solubility, due to the presence of sodium lauryl sulfate, dominates dissolution and differences in the dissolution rate are eliminated because sodium lauryl sulfate enhanced the solubility of this poorly water-soluble drug due to wetting, micellar solubilization, and/or deflocculation. However, for mebendazole the sodium lauryl sulfate present in the dissolution medium reduced the ability of the test to distinguish between the three polymorphic forms of mebendazole.

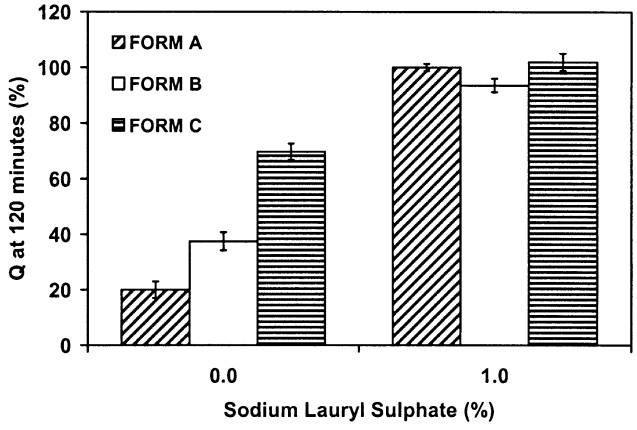

Fig. 4. Effect of 1% sodium lauryl sulfate in the dissolution medium on Q at 120 min for mebendazole polymorphs.

When sodium lauryl sulfate was removed from the dissolution medium, the percentage dissolved versus time profiles changed dramatically. Now it was clear that polymorph C went into solution faster (70% in 120 min) compared to polymorph B (37% in 120 min) and polymorph A (20% in 120 min). This order in the dissolution rate (A < B < C) does not correlate with the reported differences in solubility but does correlate with the reported in vivo effectiveness of the polymorphs [3,4,7]. This suggests that the dissolution rate of the polymorphs depended on more than just the inherent solubility of each polymorph and the degree of dispersion of the drug in the medium in which it is dissolving.

5. Summary and conclusions

Manufacturers and regulatory agencies should take care when buying or sourcing mebendazole raw material, tablets or suspensions because dissolution results obtained using the USP conditions would not ensure that the products contain the preferred polymorph C. This is important, since all three polymorphic forms of mebendazole are found in the market [10]. In developing countries such as South Africa, there are numerous generic mebendazole products available and these products are widely used because the drug forms an integral part of the essential drug list in this country. Consideration should therefore be given to

eliminating sodium lauryl sulfate from the dissolution medium for mebendazole because it will increase the ability of the dissolution test to discriminate between mebendazole polymorphs. Furthermore, other tests including IR analysis and X-ray powder diffractometry should also be used to ensure that the therapeutically preferred polymorph of mebendazole is present in drug products.

Acknowledgements

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References

[1] A.C. Evans, J.E. Finscham, M.A. Dhamsay, W. Liebenberg, Anthelmintic efficacy of mebendazole depends on molecular polymorph, S. Afr. Med. J. 89 (1999) 1118.
[2] M. Himmelreich, B.J. Rawson, T.R. Watson, Polymorphic forms of mebendazole, Aust. J. Pharm. Sci. 6 (1977) 123–125.
[3] F. Rodriguez-Caabeiro, A. Criado-Fornelio, A. Jimenez-Gonzalez, L. Guzman, A. Igual, A. Perez, M. Pujol, Experimental chemotherapy and toxicity in mice of three mebendazole polymorphic forms, Chemotherapy 33 (1987) 266.
[4] J. Costa, M. Fresno, L. Guzman, A. Igual, M. Pujol, Polymorphic forms of mebendazole: analytical aspects and toxicity, Circ. Farm. (1991) 415–426.
[5] A.A. Al-Badr, M. Tariq, Mebendazole, in: K. Florey (Ed.), Analytical Profiles of Drug Substances, Vol. 15, Academic Press, New York, 1987, pp. 292–326.
[6] W. Liebenberg, T.G. Dekker, A.P. Lötter, M.M. de Villiers, Identification of the mebendazole polymorphic form present in raw materials and tablets available in South Africa, Drug Dev. Ind. Pharm. 24 (1998) 485–488.
[7] P. Charoenlarp, J. Waikagul, C. Muennoo, S. Srinophakun, D. Kitayaporn, Efficacy of single-dose mebendazole, polymorphic forms A and C, in the treatment of hookworm and Trichuris infections, Southeast Asian J. Trop. Med. Public Health 24 (1993) 712–716.
[8] United States Pharmacopoeia, United States Pharmacopoeia, USP 25: NF 20, United States Pharmacopoeial Convention, Rockville, MD, 2002.
[9] British Pharmacopoeia, The British Pharmacopoeia, BP 2000, Her Majesty's Stationary Office, London, 2000.
[10] European Pharmacopoeia, European Pharmacopoeia, EP, 4th Edition., European Pharmacopoeia Commission, EDQM, Strasbourg, 2001.