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INVESTIGATION OF DISSOLUTION PROFILES FROM SUSPENSIONS CONTAINING BENZOYL METRONIDAZOLE USING A STATISTICAL MODEL WITH REPEATED MEASUREMENTS

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ABSTRACT

The Mexican Pharmacopeia (MP) dissolution general test was carried out on one and two lots of suspensions from each one of three commercial drug products, containing benzoyl metronidazole, and using water or 0.1N hydrochloric acid as dissolution medium. When we used paddles at 100 rpm,and water as dissolution medium, 72 to 99% of the active substance was dissolved after 60 minutes, showing differences between the dissolution profiles from these drug products. When testing conditions were changed to 0.1N hydrochloric acid at 75 r.p.m., the amount dissolved of active substance varied from 25 to 100%, showing different dissolution rate profiles for all 6 drug products.

The ANOVA for the statistical model with repeated measurements was applied to the observed dissolution rate data, showing no parallel behaviour between each dissolution profile. Using the ANOVA for nested models, and the hydrochloric acid data to investigate the dissolution efficiency, we found intraclass difference but no interclass difference. In contrast, by using the per cent dissolved

with the same dissolution medium data, we found significant differences between drug products, but not between lots.

Significant difference was observed between drug products, when using water as dissolution medium, either when using the dissolution efficiency data or the per cent dissolved in 60 minutes.

The data suggests that pharmaceutical suspensions have the same problems with the deaggregation rate such as tablets and capsules do, therefore, it is important to extend the dissolution testing for suspensions with a poor water solubility.

INTRODUCTION

Dissolution testing is a routine test for quality control to be applied to pharmaceutical solid dosage forms. However, it has been noted that heterogenous systems including suppositories and suspensions also have problems and the dissolution of their active substances are inconsistent, being the dissolution the rate-limiting step in the absorption process [1-11].

Benzoyl metronidazole is widely used as an amebicide and trichomonicide agent [12], nevertheless there is no reported dissolution testing for its suspension. For a drug product to be clinically evaluated, it requires to have optimal and confident quality standards. Thus, we were interested on developing a test for setting the dissolution profiles of benzoyl metronidazole (BM) from suspensions available in the mexican market.

In the other hand, we applied a statistical analysis for dissolution profiles using a model with repeated measurements because it is a comparative tool even more powerful than the traditional test for comparative studies for a drug dissolved expressed as per cent at each sampling time. The analysis of replicated measurements is based on the fact that each observation time applied to the same

experimental unit is not randomized. Since the 1950 decade, Box, and more recently, Greenhouse, developed this statistical model [13] and since then, it has been applied to drug dissolution profiles, specially by Mauger [14] and Fariña [15].

The purpose of this study was to establish the appropriate general conditions for determining the dissolution profiles of BM suspensions according to general guidelines for the MP dissolution general test [16]. In order to suggest an approach for routine quality control with dissolution testing of such drug products, we first applied the statistical analysis to the dissolution profile data, then the analysis for the statistical model with replicated measurements, and finally the ANOVA tests.

MATERIALS AND METHODS

Equipment. Dissolution testing assembly from Hanson-Research Model 72R. Brookfield Viscometer, Model LVF. Spectrophotometer Zeiss, Model PM2DL.

Chemicals. All used chemicals were of analytical grade. Benzoyl Metronidazole, raw material, was supplied by a national company (*Empresas R.M. de México, S.A.*).

Drug Products. We studied two lots from each one of three drug products from the mexican market; two of them containing an equivalent amount of 5 g of metronidazole (BM = 8 g), and the third one, an equivalent 2.5 g of metronidazole (BM = 4 g). The following notation was used: SA1, SA2, SB1, SB2, SC1, SC2. All this products were studied in HCl 0.IN. Only SA1, SB1,and SC1 products were studied in water.

Active Substance Content. The six BM suspensions were assayed according to the requirements of the Mexican Pharmacopeia [16]. Additionally, we determined their viscosity.

Dissolution Rate Of Benzoyl Metronidazole, Raw Material. The objective of this study was to establish more appropriate conditions, therefore we worked with two dissolution media: distilled water and 0.1N hydrochloric acid. An equivalent amount of 250 mg of metronidazole was transferred into each dissolution vessel containing 900 ml of HCl 0.I N as dissolution medium heated to 37°C. When distilled water was used, the test was performed using stirring paddles at three speeds: 60, 75, and 100 r.p.m with 90 mg of BM to avoid medium saturation [17]. When hydrochloric acid 0.1N was used, the stirring speeds were: 50, 75, and 100 rpm.

Dissolution Rates Of Benzoyl Metronidazole Suspensions. A suspension sample equivalent to 400 mg of BM (when HCl was used) or 80 mg (with water) was quantitatively transferred to the vessel bottom using a syringe, then the dissolution was carried out at the selected stirring speed. Thereafter, samples from the medium were withdrawn at 15, 30, 45, and 60 minutes. The amount dissolved was assayed spectrophotometrically with the aid of a previously validated method.

RESULTS AND DISCUSSION

Quality Control.

All drug products complied with the MP specifications except SB1, whose BM content was 86.47%. All drug products had similar viscosity.

Dissolution Rate Of Benzoyl Metronidazole, Raw Material.

Table 1 shows the percentage of BM dissolved at 60 minutes and its coefficient of variation obtained in each dissolution medium.

When distilled water was used as dissolution medium with 80 mg of BM at 60 and 75 rpm, the amount of BM dissolved was very low and, as it can be seen from the coefficient of variation (CV), the observed variation between samples was high. At 100 rpm there was an acceptable quantity of BM dissolved, having a

TABLE 1.

Disolution of benzoil metronidazole, raw material, at 60 minutes (n=6).

rpm	Water		Acid	
	% Dissolved	CV%	% Dissolved	CV%
50	--	--	61.05	17.98
60	11.94	19.94	--	--
75	14.63	21.32	93.41	7.11
100	41.67	14.53	99.05	3.69

smaller variation within vessels. Thus, this stirring speed was selected to carry out the test with all suspensions.

In 0.1N hydrochloric acid both at 75 and 100 rpm and after 60 minutes, the BM dissolved was more than 90% having small variations between samples. This is in agreement with the BM's chemical nature and its dissolution behaviour in this acid medium. In this case, the speed of 75 rpm was selected for being applied to all suspensions, not only because a high percentage of BM dissolved was obtained, but also because it was a lower stirring speed, and the dissolution profiles could be differentiated.

Dissolution of Suspensions Containing Benzoyl Metronidazole

Figure 1 shows the observed dissolution profiles of BM in water for 3 drug products. There is an apparent difference between their dissolution profiles. Also, approximately 20 to 60% general enhancement resulted in the BM dissolved from suspensions, that is, drug wetting and dissolution probably was due to excipients.

Figure 2 shows the observed BM dissolution profiles in 0.1N hydrochloric acid for all 6 drug products. The difference between them was higher. Drug product B was the limit case, having a behaviour that is unacceptable for lots of the same

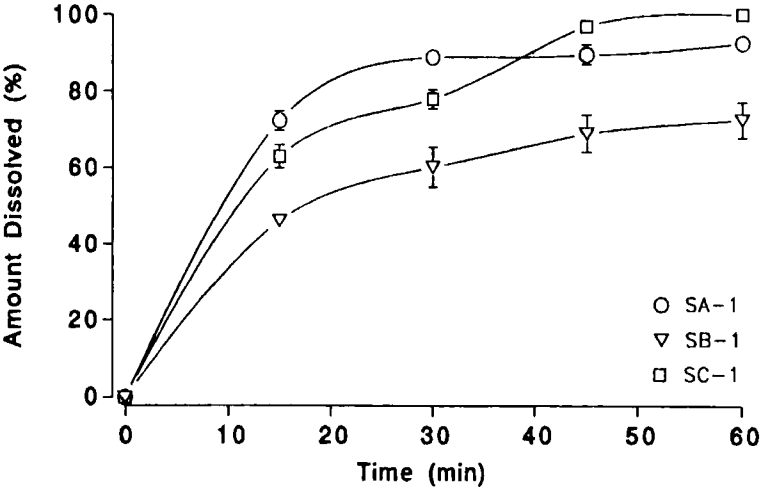

FIGURE 1.

Dissolution profiles of benzoyl metronidazole in water.

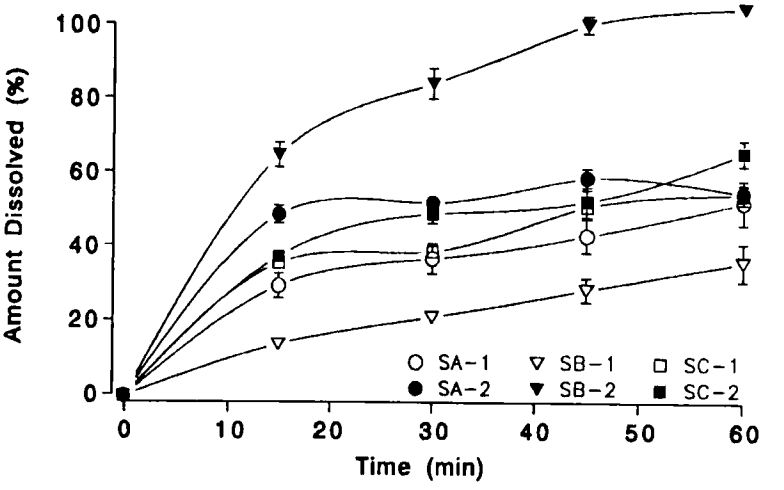

FIGURE 2.

Dissolution profiles of benzoyl metronidazole in 0.1 N hidrochloric acid.

TABLE 2.

Comparative dissolution of benzoil metronidazole at 60 minutes (n=6).

Product	Water		Acid	
	% Dissolved	CV%	% Dissolved	CV%
SA-1	92.54	5.00	52.16	28.13
SA-2	--	--	54.59	9.32
SB-1	72.53	15.79	35.97	34.27
SB-2	--	--	104.42	2.36
SC-1	99.94	5.23	54.33	12.14
SC-2	--	--	65.39	12.80

brand. In this case, the percentage of drug dissolved after 60 minutes was lower than the raw material alone. This former stands out the influence of suspending agents over drug release and dissolution behaviour in a strongly acid medium.

The comparative analysis of the summarized data of Table 2 points out that the per cent of BM dissolved after 60 minutes in water media were higher, except for SB2 suspension in acid medium, and the CV were also lower and homogenous when they are compared with those obtained in hydrochloric acid, except for SB2 suspension. This was probably due to the effect of the strong acid on suspending agents.

Statistical Analysis.

For the analysis we considered the following parameters: (a) whole dissolution-time profile [18]; (b) dissolution efficiency [19]; and (c) per cent dissolved at 60 minutes. Dissolution data from both media were used.

For the analysis of the whole dissolution-time profiles we applied the statistical model analysis with repeated measures. The significance of this statistical analysis is based on its power for entirely assessing the results obtained over

TABLE 3.

P values in ANOVA tests.

	Source of Variation	P (water)	P (acid)
Repeated measurement	Time x Lot	<0.01	<0.01
Dissolution efficiency	Brand	<0.01	NS
	Lot (within Brand)	--	<0.02
Per cent dissoved (60 min)	Brand	<0.01	<0.01
	Lot (within Brand)	--	NS

the time, and considering the dependence between them; otherwise, the statistical analysis should have been done with each one of the selected times. The statistical analysis for the profiles was performed on the data of per cent dissolved from 15 to 60 minutes. It can be seen from table 3 that with both dissolution media, there was a large time-lot interaction (TL), that indicates a lack of parallelism between profiles. This lack of parallelism prevents the source of variation from lots to be considered, therefore we proceeded making the analysis of the dissolution efficiency parameters [19] and per cent dissolved after 60 minutes. A summary with the results obtained is shown in Table 3.

The concept of dissolution efficiency [19], expressed as the per cent relationship between areas under the curve for dissolution profiles is of primary importance because it integrates the dissolution process both as amount dissolved as well as dissolution rate. Therefore, this is particularly relevant when considering pharmaceutical development or comparative research. Thus, the ANOVA of water dissolution efficiency of the three drug products, showed significant differences between them (Table 3). In the same table, there is also difference among the drug products studied when considering the percentage of BM dissolved in 60 minutes.

By considering the data obtained with hydrochloric acid medium, the nested model of ANOVA for dissolution efficiency shows significant differences within commercial brands, but not between them (Table 3). On the contrary, the statistical analysis for per cent dissolved in 60 minutes showed significant differences between commercial brands, but not between lots. This is relevant, specially regarding this test as a pharmacopoeial specification, because it guarantees the reproduction of the dissolution behaviour for drug products between lots and commercial brands.

CONCLUSION

By using BM raw material as a reference, we have been able to demonstrate that water enhanced the dissolution process of the BM suspensions studied. In contrast, the hydrochloric acid pH had influence on the formulation excipients; thus, reducing the BM dissolved.

According to the ANOVA results, water was the dissolution medium that allowed the greater difference between drug products.

The heterogenous system studied had showed problems and differences on its drug dissolution. It may be considered that in its deaggregated form, a pharmaceutical suspension has similar status as capsules and compressed tablets do; therefore, suspensions like solid dosage forms, have the same potential barriers for dissolution.

The statistical analysis for repeated measurements is appropriate for comparing parallelism between dissolution profiles as a previous step before making any comparison between lots and sampling times. In the event of a significant time-lot interaction (TL), however, the analysis of other dissolution parameters should be performed including dissolution efficiency or percentage of drug dissolved at a given time.

It is hoped that future research of this kind will focus on elucidation of the dissolution profiles, thus setting the *in vitro* conditions as well as a comparative pattern for this test. From the point of view of a physico chemical quality control, this will guarantee a consistent behaviour, which in turn is a prerequisite for an *in vivo* assessment.

The dissolution test using No. 2 MP apparatus at 100 rpm and water as medium, may be used for stablishing the dissolution testing of BM suspensions as an applicable pharmacopoeial test.

The differences observed in the dissolution profiles may be caused by factors including particles suspended of different size, drug-excipient complexes formation, changes in the microenvironmental viscosity with pH, among others. In the next future, we will study some of these factors.

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