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RESEARCH PAPER

Comparison of Dissolution Profiles for Albendazole Tablets Using USP Apparatus 2 and 4

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ABSTRACT

The in vitro dissolution of albendazole from three different commercially available products (200 mg tablets) was studied using U.S. Pharmacopeia (USP) Apparatus 2 and USP Apparatus 4 in order to compare the release performance of the drug in two essentially different dissolution systems. For both cases, 0.1 N HCl was used as dissolution medium. Only the reference product and one of the generic products studied met the 80% USP 24 specification for albendazole dissolved at 30 min, using USP Apparatus 2. Although the reference product reached 80% of albendazole dissolved at 30 min when Apparatus 4 was used, the generic products' dissolution performance was markedly reduced in this system. Though dissolution rate was slower using Apparatus 4, the total quantity of albendazole dissolved from the reference product, represented by area under the dissolution profile, was practically the same regardless of the system used. Dissolution kinetics of albendazole was adequately described by Weibull's function for all the products. The dissolution time (t_d) derived from data fitting to this function showed significant differences among the products studied. Data analysis based on analysis of variance (ANOVA) showed nonequivalence among the dissolution profiles of generic products compared with the reference product either with the dissolution vessel system or the flow-through cell, as well as nonequivalence among the dissolution profiles using both apparatuses with the same product. Though differences in the dissolution profiles for generic products against the reference product in both systems were found, USP Apparatus 4 showed higher discriminative capacity in differentiating the release characteristics of the products tested.

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Key Words: Albendazole; Generic products; Dissolution profiles; Flow-through cell apparatus.

INTRODUCTION

In vitro dissolution specifications are established to ensure batch-to-batch consistency while pointing out any potential problems with in vivo bioavailability among different drug products. The characteristics of a dissolution test should be developed while taking into account drug solubility, pKa/pH ratio, $K_{octanol/water}$, and drug permeability.[1]

The Biopharmaceutics Classification System (BCS) can be used as a basis for setting in vitro specifications[2] and can also provide a basis for predicting the probability of achieving a successful in vitro–in vivo correlation (IVIVC). In the case of low solubility/high permeability drugs (Class 2), drug dissolution might be the rate-limiting step for drug absorption, and IVIVC may be expected. Therefore, it is quite important to pay attention to the design of a dissolution test for these drugs, especially for the proper choice of media ingredients, hydrodynamic characteristics, and the testing time.

The ability to test whether the product is able to release an active substance within an expected time period under physiological conditions can be a difficult task by using common apparatus. The probability of establishing a significant correlation will greatly depend on the success of obtaining in vitro conditions quite close to the in vivo conditions of a relevant drug.[3] Class 2 drugs' absorption is faster than dissolution, so it is feasible that sink conditions will also prevail in vivo. Thus, it is necessary to hold these conditions throughout the in vitro study.

Albendazole is a broad-spectrum antihelmintic drug with systemic action and scarce solubility.[4,5] Absorption of albendazole occurs along the gastrointestinal tract, and it has been shown that solubility and not permeability is the limiting step in the absorption of the drug.[5] Therefore albendazole is a Class 2 drug according to BCS (low solubility/high permeability). The U.S. Pharmacopeia (USP) 24 dissolution method for albendazole tablets includes Apparatus 2 at 50 rpm and 900 mL of 0.1 N HCl as dissolution medium.[6] Under thse conditions, not less than 80% of albendazole must be dissolved at 30 min ($Q = 80\%$). The flow-through cell (Apparatus 4) has been recently introduced into USP dissolution methods, and several advantages in testing low-soluble drugs, along with findings in establishing adequate IVIVC, have been attributed to it.[7] So, it would be important to investigate the dissolution performance of albendazole tablets in this system and compare it to Apparatus 2.

The objective of the present study was to evaluate an alternative dissolution method for albendazole tablets, using a flow-through cell apparatus compared with the official one (Apparatus 2).

MATERIALS AND METHODS

Solubility of Albendazole in HCl 0.1 N

The solubility of albendazole in 0.1 N HCl at 37°C was determined using Albendazole reference standard (ABA-1 lot, Secretariat of Health, Mexico). The test was performed in duplicate and under constant stirring for 16 h; samples were filtrated and the quantity of drug dissolved was determined spectrophotometrically at 291 nm.

Dissolution Studies

Three 200 mg albendazole products (tablets) produced in Mexico, were directly purchased at the pharmacy: Zentel lot 61006, SmithKline Beecham ("A", reference product), Digezanol lot 002136, Hormona and Bendapar lot 008035, Fustery ("B" and "C", generic products). All the products were tested for assay and content uniformity according to USP 24.

Dissolution studies on albendazole tablets were conducted in USP Apparatus 2 (paddle method), Hanson Research 72 RL, according to USP 24 monograph. Twelve replicates for each product were used. The dissolution medium used was 900 mL of degassed 0.1 N HCl at 37° ± 0.5°C. The paddle rotation speed was 50 rpm. In all experiments, 3 mL of dissolution samples were withdrawn at previously selected times during 60 min without replacement of medium. Samples were filtered through 0.45 μm membranes and assayed by a spectrophotometric method at 291 nm (UV/VIS Perkin Elmer Lambda-10S spectrophotometer). For every trial, a standard curve was prepared. Cumulative percentages of drug release from the dosage forms were calculated based on drug content for each product.

Additionally, a dissolution study was performed on albendazole products using USP Apparatus 4 (flow-through cell method), Sotax CH4123

automatized dissolution tester, with cells of 22.6 mm (i.d.), coupled to a UV/VIS Perkin Elmer Lambda-10S spectrophotometer. The degassed dissolution medium, 0.1 N HCl at $37 \pm 0.5^{\circ}C$, was pumped at a flow rate of 16 mL/min. An open system was used, without recycling the dissolution media. Twelve tablets of each product were tested. The tablets were positioned directly onto a bed of 6 grams of glass beads (0.75–1 mm), thus filling the conical part of the bottom of each cell. The quantity of albendazole dissolved at previously selected times was determined by a spectrophotometric method at 291 nm. For every trial, a standard curve was prepared.

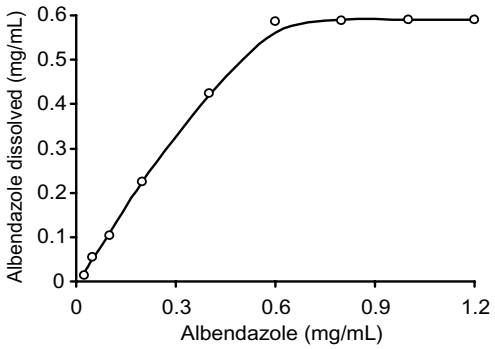

Figure 1. Albendazole solubility in 0.1 N HCl, at 37°C.

Data Analysis

In order to compare the dissolution profiles of albendazole products, two model-independent methods were used: area under dissolution curve (AUC), obtained by trapezoidal rule[8] and Moore's similarity factor f_2.[9] The AUC values were compared by one-way analysis of variance (one-way ANOVA) followed by Dunnett's test.

A model-dependent method was also used, fitting dissolution data to different dissolution kinetic functions: zero order, first order, cubic root law (Hixson-Crowell), square root of time equation (Higuchi), and Weibull's equations.[10,11] Nonlinear regression to fit the data was used; standard criteria, i.e., higher determination coefficient (r^2), and smaller residual mean square (RSM), were used to select the best model. Mean parameters derived from the best fit model for the different products data were compared by two-way analysis of variance (two-way ANOVA). Comparison of dissolution profiles of albendazole from generic products against reference product was also performed by multivariate analysis of variance (MANOVA) upon repeated measures design.[10,12] In addition, a univariate one-way ANOVA was conducted in order to compare the percentage of albendazole dissolved at each time point, followed by Dunnett's test for multiple comparisons. Data analysis was carried out using SPSS software (Version 10.0). Differences were considered significant if $p < 0.05$.

RESULTS AND DISCUSSION

Albendazole Solubility

Albendazole solubility data determined in 0.1 N HCl at 37°C is shown in Fig. 1. As can be seen, the saturation concentration is about 0.6 mg/mL. Assuming that albendazole belongs to Class 2 (low solubility/high permeability), it would be desirable to conduct the dissolution study of albendazole tablets under sink conditions. Theoretically, the pharmacopoeial dissolution test of albendazole tablets does not comply with such conditions since it would be necessary to hold the drug concentration in the dissolution medium under 10% of the saturation concentration (< 0.1 Cs), that is less than 0.06 mg/mL (54 mg of albendazole in 900 mL of medium). The dissolution medium volume required to maintain sink conditions for a 200 mg albendazole dose would be more than 3 L. On the contrary, it would be expected that a continuous change of the dissolution medium in the flow-through cell system would hold sink conditions throughout the study,[13,14] similar to those encountered in vivo.

Dissolution Studies

Weight variation, drug content, and content uniformity test results satisfied the pharmacopoeial specifications for all the products.

The dissolution profiles obtained using USP Apparatus 2 and USP Apparatus 4 are shown in Fig. 2. Differences in dissolution behavior of generic products B and C compared with reference product were observed and even greater when USP Apparatus 4 was used.

The percent of albendazole dissolved at 30 min for each product, using USP Apparatus 2 and 4 is shown in Table 1. Considering a single-point specification at 30 min ($Q = 80\%$, USP Apparatus 2), some assertions can be worked out: product A meets the minimum specification of 80% dissolved in the two

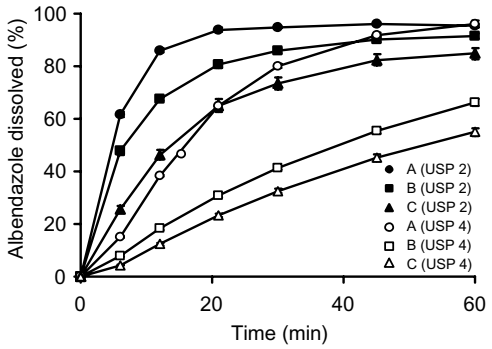

Figure 2. Dissolution profiles for albendazole generic (B, C) and reference (A) products in USP Apparatus 2 and 4. mean ± SEM, $n = 12$.

Table 1. Dissolution parameters for albendazole generic (B, C) and reference (A) products in USP Apparatus 2 and 4 Apparatus. Mean ± SEM, $n = 12$.

Apparatus	Product	% Dissolved at 30 min	ABC (% min)
USP 2	A	94.8 ± 0.50	4565 ± 99
	B	86.0 ± 0.98	4335 ± 134
	C	73.5 ± 2.37[a]	3584 ± 171
USP 4	A	80.1 ± 0.93	4392 ± 252
	B	41.2 ± 0.65[a]	2130 ± 110
	C	32.6 ± 0.88[a]	1489 ± 64

[a] $P < 0.05$ vs. A, Dunnett's test.

dissolution test systems, whereas product C did not meet the specification in either of the two systems. Nevertheless, the results of product B are contradictory between both apparatuses. Considering a 30 min time point in Apparatus 2, product B would be approved, but this would not be the case using Apparatus 4 under the same criterion.

Though dissolution rate was slower in the assembly of USP Apparatus 4 for all products, the reference product reached a high percentage dissolved after 30 min (≥ 80%) and thoroughly dissolved at 60 min (100%). This was not the case with generic products, for which only about 70% of active substance was dissolved at the same time. The slower dissolution rate observed in USP Apparatus 4 can be explained by differences in hydrodynamic conditions that characterize these systems. Apparatus 4 utilizes no stirring mechanism, so the dosage form and drug particles are exposed continuously to homogeneous, nonturbulent, laminar flow, causing a slow dissolution rate. On the other side, in the beaker method the turbulent solvent flow associated with stirring mechanisms imparts variable degrees of physical abrasion of the solids, due to nonhomogeneous shear rate of transfer over the surface of the particles, thus enhancing the dissolution rate.[15] Nevertheless, the continuous media restoration in USP Apparatus 4 allowed the maintenance of albendazole concentration in the medium under the saturation limit (< 0.1 Cs), i.e., under sink conditions, and complete dissolution of albendazole from the reference product at 60 min was attained.

Independent-Model Method

In order to compare the dissolution profiles of albendazole products studied, two model-independent methods were used: area under dissolution curve (AUC) and Moore's similarity factor (f_2).

The ultimate challenge to dissolution testing is its ability to reflect the in vivo performance of the dosage unit during its absorption phase. Numerous variables have been derived from in vitro data, which have been correlated with in vivo data. In vitro dissolution profiles can statistically be compared through mean dissolution time, area under dissolution curve.[12,16] In the present study AUC was considered a more reliable parameter to estimate the extent of drug dissolved from the dosage form in vitro because this parameter can probably provide a better indication of in vivo performance.[16] Values for AUC calculated from dissolution data of products are shown in Table 1. Analysis of variance showed significant differences in AUCs for the products studied ($p < 0.001$). When Apparatus 2 was used, only the AUC generic product B was equivalent to the AUC of reference product ($p > 0.05$), while in USP Apparatus 4 the AUCs of both generic products (B and C) showed significant differences against the reference ($p < 0.001$). These results demonstrated that USP Apparatus 4 had a better discriminative capacity in differentiating the release characteristics of the albendazole products tested. In addition, when the AUCs obtained for each product in the two systems were compared, no differences were found for the reference product A, while the AUCs for generic products varied widely (Table 1).

Differences between the dissolution profiles of generic albendazole products and the reference product were also found by the similarity Moore's factor ($f_2 < 50$) in both dissolution systems (Fig. 3). Using Apparatus 2, f_2 values were 46.4 and 28.2 for

product B and C respectively; when Apparatus 4 was used, differences were more pronounced and f_2 values were 26.3 and 21.1 for products B and C respectively. Moore's similarity factor f_2 has been endorsed by the Food and Drug Administration (FDA) as an acceptable method for dissolution profiles comparison.[1,17] The above results showed again that USP apparatus 4 is a more sensitive method to distinguish the dissolution profile among different albendazole products.

Dependent-Model Method

In order to describe the albendazole release kinetics from products studied, dissolution data were fitted to various kinetics dissolution models: zero-order, first-order, Hixson-Crowell, Higuchi, and Weibull's equations.[11–13] The results are summarized in Table 2. As can be seen, Weibull's function fitted best to the dissolution data for all the products in the two dissolution systems ($r^2 > 0.99$ and minimum residual mean square, RMS). This shows that generic products exhibited the same kinetic performance as the reference product. The results agreed with those obtained by other authors that have pointed out that Weibull's function appears to be one of the better methods to fit the different types of dissolution profiles.[11,12]

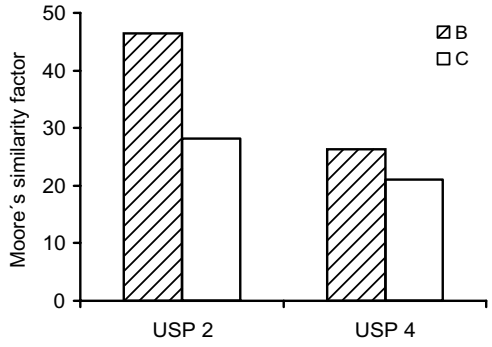

Figure 3. Moore's similarity factor for albendazole generic products.

The comparison of dissolution profile using a dependent-model method was made by analyzing the derived parameter (t_d) from Weibull's function expressed as:

$$Q(t) = 1 - e^{(t/t_d)^{\beta}}$$

where $Q(t)$ is the percentage dissolved at time t, t_d is the mean dissolution time (scale parameter) and β is the shape parameter. In the results shown in Fig. 4, parameter (t_d) is equivalent to mean dissolution time (TMD) calculated by statistical moments. The values of t_d were compared by one-way ANOVA followed by Dunnett's test. Significant differences in t_d between generic products and the reference product were found in both apparatus ($p < 0.001$).

ANOVA-Based Method

Finally, ANOVA-based methods were used to compare the albendazole products tested. The advantages of using ANOVA is that it is not restricted to any of the requirements suggested for Moore's factor f_2 and does not depend on data fitting to a specific kinetic model. Since the data were collected as

Table 2. Criteria used for the selection of the best kinetic model.

	Apparatus	Product	Zero order	First order	Hixson Crowell	Higuchi	Weibull
Determination coefficient	USP 2	A	0.4647	0.9887	0.8760	0.7545	0.9925
		B	0.6237	0.9617	0.9203	0.8777	0.9951
		C	0.7978	0.9786	0.9512	0.9569	0.9913
	USP 4	A	0.8686	0.9846	0.9906	0.9516	0.9970
		B	0.9776	0.9964	0.9956	0.9550	0.9992
		C	0.9870	0.9962	0.9964	0.9321	0.9975
Residual mean square	USP 2	A	807.61	16.71	186.53	370.12	11.12
		B	493.80	48.09	101.57	160.34	6.32
		C	250.98	23.34	55.06	54.32	9.72
	USP 4	A	226.80	27.53	15.91	84.05	4.85
		B	15.40	2.63	2.81	33.76	0.53
		C	6.74	2.06	1.82	35.31	1.23

repeated measurements over time on the same experimental unit, a repeated measures analysis was performed in order to compare the complete dissolution profiles for the generic albendazole products to that of the reference product, as well as to compare dissolution profiles for the same product using both USP Apparatus 2 and 4. Because cumulative release data at each time point are intercorrelated, the multivariate approach (MANOVA) was used on crude original data.[11] The hypothesis on time × product interaction effect, which is interpreted as parallelism or shape of the profiles, indicating that the difference of percent dissolved between two products is constant at any two points of time considered, was rejected, i.e., dissolution profiles were not parallel (Table 3). Wilk's lambda values indicated significant differences between the shape of the dissolution profiles of the generic products and the reference product, in both apparatuses. As can be seen, Wilk's lambda values for USP Apparatus 4 were smaller than the values for USP Apparatus 2, thus indicating greater differences in their dissolution profiles shape. Also, nonparallelism among dissolution profiles in both apparatuses for the same products was observed. The same conclusions were derived using other tests, such as Pillai's trace, Hotelling's trace and Roy largest root test.

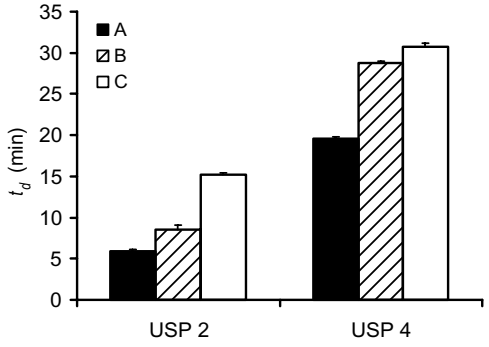

Figure 4. Albendazole t_d derived from Weibull's function. Bars represent the mean ± SEM, $n = 12$.

Table 3. Time × product interaction effect obtained from MANOVA of albendazole dissolution profiles.

Apparatus	Comparison	Wilk's lambda	p
USP 2	B vs. A	0.0295	<0.001
	C vs. A	0.0146	<0.001
USP 4	B vs. A	0.0095	<0.001
	C vs. A	0.0060	<0.001

As nonparallelism of the dissolution profiles was found, a univariate analysis for the percentage dissolved at each time point data was performed using one-way ANOVA followed by Dunnett's test, in order to find the source of difference. Significant differences were observed at most time–points, except at 60 minutes for product B using Apparatus 2 (Table 4).

Finally, both statistical analysis (MANOVA and ANOVA) emphasized the greater sensitivity and discriminative capacity of the USP Apparatus 4 in the evaluation of dissolution performance of albendazole tablets in comparison to the official method (Apparatus 2). Sensitivity, discriminating ability, and reproducibility are important qualifications for a dissolution method. The FDA's dissolution

Table 4. Multiple comparisons (Dunnett's test) for the percentage of albendazole dissolved at each time-point from generic products vs. reference product in USP Apparatus 2 and 4.

		USP Apparatus 2		USP Apparatus 4	
Comparison	Time (min)	Difference	p	Difference	p
B vs. A	6	13.99	<0.001	7.17	<0.001
	12	18.41	<0.001	19.90	<0.001
	21	13.32	<0.001	34.01	<0.001
	30	8.72	<0.050	38.93	<0.001
	45	6.02	<0.050	36.28	<0.001
	60	3.85	<0.050	29.70	<0.001
C vs. A	6	36.06	<0.001	10.72	<0.001
	12	39.59	<0.001	26.04	<0.001
	21	28.77	<0.001	41.60	<0.001
	30	21.30	<0.001	47.51	<0.001
	45	13.82	<0.001	46.47	<0.001
	60	10.60	<0.001	41.04	<0.001

guidelines identify the need for a sensitive test, because this will allow detection of in vitro differences before the in vivo performance is impacted.[1] In the present study, USP Apparatus 4 was a very sensitive and discriminative system; however, one should not forget that the primary objective in designing a dissolution test is to reflect the in vivo behavior of a drug product. The study revealed significant differences in the dissolution behavior between generic albendazole products and the reference product, but the relation of these differences with in vivo behavior has not been demonstrated yet. Nevertheless, albendazole characteristics (low solubility/high permeability), together with the data obtained in this study, suggest the performance of an in vivo assessment.

CONCLUSIONS

Significant differences between albendazole dissolution profiles for generic products and the reference product, using either USP Apparatus 2 or USP Apparatus 4 were found. There were also differences among the profiles obtained using USP Apparatus 2 and USP Apparatus 4 with the same drug product. The total amount of albendazole dissolved from the reference product was practically the same regardless of the system used.

All methods applied to comparison of dissolution profiles demonstrated a greater sensitivity and discriminative capacity of USP Apparatus 4 in detecting differences of the dissolution behavior of the albendazole products studied.

Further in vivo bioavailability studies should be conducted in order to confirm any correlation with the in vitro performance of albendazole products.

REFERENCES

1. FDA. *Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms*; U.S. Department of health and human services, FDA. Center for Drug Evaluation and Research: Rockville, MD, August 1997.
2. Amidon, G.L.; Lennernas, H.; Shah, V.P.; Crison, J.R. A theoretical basis for a biopharmaceutical drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. **1995**, *12* (3), 413–420.
3. Dressman, J.B.; Amidon, G.L.; Reppas, C.; Shah, V.P. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm. Res. **1998**, *15* (1), 11–22.
4. Del Brutto, O.H. Medical treatment of cysticercosis–effective. Arch. Neurol. **1995**, *52* (1), 102–104.
5. Jung, H.; Medina, L.; García, L.; Fuentes, I.; Moreno-Esparza, R. Absorption studies of albendazole and some physicochemical properties of the drug and its metabolite albendazole sulphoxide. J. Pharm. Pharmacol. **1998**, *50*, 43–48.
6. U.S. Pharmacopoeial Convention. The United States Pharmacopoeia, 24th rev., Rockville, MD, 2000, 52–54.
7. Lootvoet, G.; Beyssac, E.; Shiu, G.K.; Aïache, J.M.; Ritschel, W.A. Study on the release of indomethacin from suppositories: in vitro-in vivo correlation. Int. J. Pharm. **1992**, *85*, 113–120.
8. Rowland, M.; Tozer, T.N. *Clinical Pharmacokinetics. Concepts and Applications*, 3rd Ed.; Lea & Febiger: New York, 1995; 469–472.
9. Moore, J.W.; Flanner, H.H. Mathematical comparison of dissolution profiles. Pharm. Technology **1996**, *20* (6), 64–75.
10. Sathe, P.M.; Tsong, Y.; Shah, V.P. In vitro dissolution profile comparison: statistics and analysis, model dependent approach. Pharm. Res. **1996**, *13*, 1799–1803.
11. Yuksel, N.; Kanik, A.E.; Baykara, T. Comparison of in vitro dissolution profiles by ANOVA-based, model-dependent and independent methods. Int. J. Pharm. **2000**, *209* (1–2), 57–67.
12. Polli, J.E.; Rekhi, G.S.; Augsburger, L.L.; Shah, V.P. Methods to compare profiles and a rationale for wide dissolution specifications for metoprolol tartrate tablets. J. Pharm. Sci. **1997**, *86* (6), 690–700.
13. Langenbucher, F.; Benz, D.; Kürth, W.; Möller, H.; Otz, M. Standardized flow-cell method as an alternative to existing pharmacopoeial dissolution testing. Pharm. Ind. **1989**, *51* (11), 1276–1281.
14. Qureshi, S.A.; Caillé, G.; Brien, R.; Piccirilli, G.; Yu, V.; Mc Gilveray, I.J. Application of flow-through dissolution method for evaluation of oral formulations of nifedipine. Drug. Dev. Ind. Pharm. **1994**, *20* (11), 1869–1882.

15. Abduo, H.M. *Dissolution*, *Bioavailability* & *Bioequivalence*; Mack Pub. Co.: Easton, Pennsylvania, 1989; 128–129.
16. Banakar, U.V. Dissolution & bioavailability. In *Pharmaceutical Dissolution Testing*; Banakar, U.V., Ed.; Marcel Dekker, Inc.: New York, 1992; Vol. 49, 347–384.
17. Shah, V.P.; Tsong, Y.; Sathe, P.; Liu, J.P. In vitro dissolution profile comparison-statistics and analysis of the similarity factor, f_2. Pharm Res. **1998**, *15* (6), 889–896.
18. Polli, J.E.; Rehki, S.; Saha, V.P. Methods to compare dissolution profiles. Drug Int. J. **1996**, *30*, 1113–1120.