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Importance of using physiologically relevant volume of dissolution medium to correlate the oral exposure of formulations of BMS-480188 mesylate

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Abstract

BMS-480188 is a weak base. The aqueous solubility of BMS-480188 is 0.036 mg/ml at pH 6.5 at 37 °C. The mesylate salt of BMS-480188 was prepared to improve its solubility. Capsules containing mesylate salt alone (Formulation A) or mesylate salt with excipients, including lactose, croscarmellose sodium, sodium lauryl sulfate, syloid and magnesium stearate (Formulation B), were prepared. Both formulations show similar dissolution profiles in 1 l 0.01N HCl at 37 °C. However, the bioavailability of Formulations A and B is 5.7 and 24%, respectively, in monkeys. Since very small amount of fluid is available in the stomach of monkeys in fasted state, 30 ml of 0.01N HCl was used as the dissolution medium to simulate the ratio of the drug to dissolution medium in vivo. The dissolution studies in 30 ml of 0.01N HCl show that the amount of drug dissolved from the Formulation B is 80% greater than the Formulation A after 2 h. These results are consistent with the higher bioavailability of the formulated capsules. The pK_a of the free base is 3.0 and the apparent solubility of the mesylate salt (>20 mg/ml) is much greater than the equilibrium solubility of BMS-480188 (1.08 mg/ml) in 0.01N HCl at 37 °C. Therefore, the mesylate salt of BMS-480188 converts to the free base in 0.01N HCl. The presence of excipients delays the conversion of the mesylate salt to the free base in the dissolution test using 30 ml medium, leading to a greater percentage of the dissolved drugs. This inhibitory effect of excipients is masked during the dissolution using 1 l medium because the concentration of the dissolved drug is below the solubility limit of BMS-480188. This study demonstrates the importance of the volume of the dissolution medium for the in vitro dissolution test to qualitatively predict the bioavailability of a salt of weak base with low intrinsic aqueous solubility.

Keywords: Bioavailability; BMS-480188; Dissolution; Formulation; Volume of dissolution medium

1. Introduction

In pharmaceutical field, the dissolution test is widely used to evaluate various formulations and predict their bioavailability (Amidon et al., 1995; Dressman et al., 1998; Galia et al., 1998). A typical

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USP dissolution test employs 900 ml of the dissolution medium. Sink conditions are maintained during dissolution. However, during in vivo dissolution, the sink conditions may not exist, especially for those drugs with low aqueous solubility. Both composition and volume of the dissolution medium have been shown to be critical in correlating the results of in vitro dissolution test with bioavailability (Dressman and Reppas, 2000). Since the volume of gastric fluid available in animal species or humans for dissolution is significantly lower than 900 ml, a dissolution test that uses a smaller and physiologically relevant volume of medium is more likely to qualitatively predict the in vivo performance of a formulation, particularly for weak bases with low intrinsic aqueous solubility.

In this study, we report a case that demonstrates the importance of the volume of medium in dissolution test to qualitatively predict the bioavailability of formulations. The results of dissolution tests of two formulations using a small rather than a ubiquitous large volume of dissolution medium qualitatively correlated with the bioavailability of the mesylate salt of BMS-480188 in monkeys.

2. Materials and experiments

2.1. Materials

The mesylate salt of BMS-480188 (purity > 99.8%) was provided by Process R&D at Bristol-Myers Squibb Co. Polymorph I of the free base was obtained by suspending the salt in 1 mg/ml povidone (MW 40K) aqueous solution for 10 min, followed by filtration and air drying. Polymorph II of the free base was prepared by suspending the salt in water for 24 h followed by filtration and air-drying.

Excipients, including anhydrous lactose (Foremost Farm USA, Baraboo, WI), fast flow lactose (Foremost Farm USA, Baraboo, WI), microcrystalline cellulose (FMC, Newark, DE), croscarmellose sodium (FMC, Newark, DE), sodium lauryl sulfate (J.T. Baker, Phillipsburg, NJ), and magnesium stearate (Mallinckrodt, Hazelwood, MO) were used for preparing capsule formulations. Empty hard gelatin capsules (size #0) were purchased from Capsugel (Greenwood, SC). 1N hydrochloric acid was purchased from EM Science (Gibbstown, NJ). Water was purified by Milli-Q Plus^{TM} system (Millipore Co., Bedford, MA).

2.2. Capsule preparation

The mesylate salt of BMS-480188 and excipients were screened through a #30 mesh sieve. The sieved salt was mixed with excipients listed in Table 1 except magnesium stearate in a high shear mixer (Model KG5, Key International, Inc.) for 3 min. The mixture was then blended with magnesium stearate in the Key mixer for 30 s. The capsules were prepared by hand filling the salt or the blend into #0 hard gelatin capsules. The compositions of Formulations A–D are presented in Table 1.

2.3. Physicochemical characterization

The pH–solubility profile of BMS-480188 at the room temperature (24 ± 3 °C) was determined (Fig. 1). Excess solids were suspended in 10 ml of aqueous solution with adjusted pH and the suspension was equilibrated by shaking in a water bath for 48 h at the designated temperature. Aliquots were withdrawn and filtered through 0.45 μm filters. The filtered solution was diluted with acetonitrile and was analyzed by HPLC. The solubility of BMS-480188

Table 1
Compositions of various formulations of the mesylate salt of BMS-480188

Formulation	BMS-480188 mesylate (%)	Lactose (anhydrate) (%)	Lactose (fast flow) (%)	Microcrystalline cellulose (%)	Croscarmellose sodium (%)	Sodium lauryl sulfate (%)	Syloid (%)	Magnesium stearate (%)
A	100.0	–	–	–	–	–	–	–
B	52.1	20.2	20.2	–	4.0	1.0	2.0	0.5
C	52.1	20.7	20.7	–	4.0	–	2.0	0.5
D	52.1	–	–	41.4	4.0	–	2.0	0.5

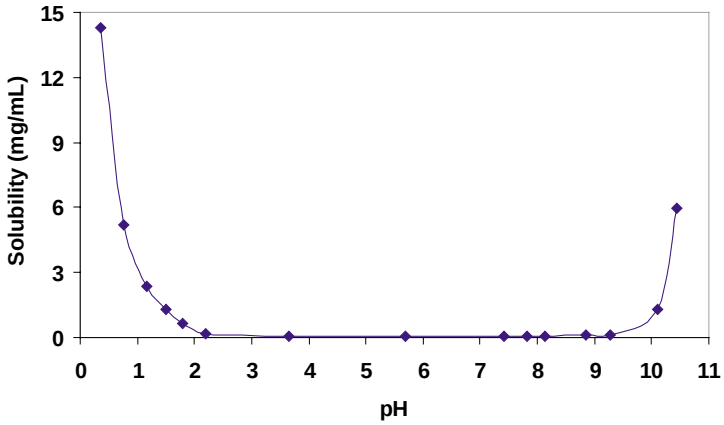

Fig. 1. pH–solubility profile of BMS-480188 at room temperature (24 ± 3 °C).

alone or in the presence of excipients was also determined in 0.01N HCl at 37 °C using the above procedure.

Powder X-ray diffractometer (Miniflex, Rigaku, Japan) was used to identify the polymorph of the free base or the mesylate salt. The samples were exposed to Cu Kα radiation (35 kV and 30 mA) and were scanned from 5 to 35° 2θ at a step size of 0.01° and at 1 s per step.

The thermal properties of BMS-480188 were determined by differential scanning calorimetry (TA Instrument, New Castle, DE). The temperature axis and the cell constant were calibrated with indium (99.9% pure). The sample was placed in an open aluminum pan and was scanned from room temperature to 300 °C at 10°C/min with nitrogen purge at 100 ml/min.

2.4. High performance liquid chromatography (HPLC)

The concentration of BMS-480188 was determined by HPLC using an Alliance™ system (Waters Inc., Milford, MA). Luna C18 column (150 mm × 4.6 mm, 5 μm particle size, Phenomenex, Torrance, CA) was used. The mobile phase consisted of 30% acetonitrile and 70% 0.1 M ammonium acetate aqueous solution. The flow rate was set at 1.0 ml/min and the wavelength of the UV detector was 254 nm.

2.5. Dissolution test

Dissolution studies were conducted using a Distek Dissolution System (Model 2100C, North Brunswick, NJ) at 37 °C. Either 1 l or 30 ml of 0.01N HCl was used as the dissolution medium. The dissolution medium was stirred using a paddle at 50 rpm. At designated intervals, 1 ml (for 30 ml dissolution medium) or 5 ml (for 1 l dissolution medium) of the test solution was withdrawn and the concentration of BMS-480188 was determined by HPLC or by automatic sampling UV detector (Hewlett-Packard, Palo Alto, CA). The concentration determined by the automatic sampling UV detector was validated by HPLC.

2.6. Solution mediated conversion of the mesylate salt of BMS-480188 to free base

Solution mediated conversion of the mesylate salt of BMS-480188 to the free base was studied and the effect of excipients on the precipitation rate of the free base was examined. A volume of 10 ml of 0.01N HCl (37 °C) was added to 100 mg of the mesylate salt of BMS-480188, or 192 mg Formulation B containing 100 mg mesylate salt, or 192 mg Formulation C containing 100 mg mesylate salt. The suspension was vigorously shaken by vortex for 20 s and the solution was filtered. The filtered solution was placed in a water bath at 37 °C and was shaken at approximately 60 strokes/min. The precipitation of the free base from

the solution was monitored. A portion of the suspension was withdrawn and filtered at designated times and the concentration of BMS-480188 in the filtrate was determined by HPLC. In addition, the solid phase in the suspension was collected and characterized by powder X-ray diffractometer.

2.7. Bioavailability study

The pharmacokinetics and absolute bioavailability of BMS-480188 mesylate were evaluated in three male cynomologus monkeys. The monkeys were administered a single capsule containing 62.5 mg of the mesylate salt of BMS-480188 (Formulation A), a single capsule containing 62.5 mg of the BMS-480188 mesylate salt with excipients (Formulation B), and a single intravenous 15 min infusion of a solution of BMS-480188 formulated in 90% sodium phosphate buffer (25 mM)/5% ethanol/5% Tween 80®, at 5 ml/kg, according to a three-period, non-randomized, three-way crossover design. A fixed volume of water (15 ml) was given to the monkeys after capsule administration. A washout period of at least 3 days was allowed between consecutive doses. Blood samples were collected at 0, 5, 15, and 30 min, and at 1, 2, 4, 6, 8, and 24 h after intravenous administration. Blood samples were collected at 0, 15, 30 min, and at 1, 2, 4, 6, 8, and 24 h following oral administration. The plasma samples were analyzed for BMS-480188 by a validated LC/MS/MS method with a detection limit of 1 ng/ml.

3. Results and discussion

3.1. Physicochemical properties of BMS-480188 mesylate

BMS-480188 is a weak base with a pK_a of 3.0. Polymorph I is less stable than polymorph II at room temperature. The pH–solubility profile of BMS-480188 is shown in Fig. 1, which indicates that the pH_{max} of BMS-480188 in HCl is lower than 1.0. During solubility studies in 0.1 or 0.01N HCl, the equilibrium solid phase is found to be the free base. This is because the solution pH is higher than pH_{max}, in which the free base has the lower solubility than the salt. The solubility of the free base in water is enhanced by salt formation. The apparent solubility of the mesylate salt of BMS-480188 in water is >20 mg/ml. However, when the mesylate salt of BMS-480188 is suspended in water or HCl solutions (0.1N or 0.01N), it converts to the free base through a solution mediated transformation because of significantly lower solubility of BMS-480188. During this transformation, polymorph I, which is a metastable form of the free base, precipitated along with the more stable polymorph II. Within minutes, the metastable polymorph I then transforms to polymorph II in the presence of water. The solubility of polymorph II in 0.01N HCl at 37 °C (pH 1.8) is 1.08 mg/ml. The solubility of BMS-480188 is not affected by the presence of excipients used in Formulation B. Due to rapid transformation in the aqueous solution, the solubility of polymorph I cannot be accurately determined.

3.2. Dissolution and conversion of the mesylate salt of BMS-480188 in 0.01N HCl

The dissolution profiles of Formulations A–D in 1 l dissolution medium are shown in Fig. 2. The profiles indicate that Formulations A and B show similar dissolution profile while the dissolution of Formulation D is much slower. The slower dissolution of Formulation D is attributed to the formation of a plug around the insoluble excipient, microcrystalline cellulose (MC). The mesylate salt exhibits high apparent solubility in 0.01N HCl (>20 mg/ml), followed by rapid precipitation of free base. In the presence of MC, the diffusion of the dissolved BMS-480188 is inhibited, leading to its high local concentration. The free base then precipitates around MC to form a plug. Because the dissolution rate of BMS-480188 free base is very slow in 0.01N HCl (Fig. 4), the formation of free base inhibits further dissolution of the remaining undissolved salt.

Based on the dissolution results in 1 l 0.01N HCl, it was expected that Formulations A and B would show similar in vivo bioavailability. However, in monkeys, the bioavailability of Formulation B is 24% while that of Formulation A is only 5.7% (Table 2). Obviously, the dissolution test in 1 l 0.01N HCl failed to predict the in vivo performance of Formulations A and B. Considering the fact that the volume of gastric fluid in the stomach in the fasted state is very small, a dissolution study was conducted using 30 ml dissolution medium with a ratio of drug to volume of

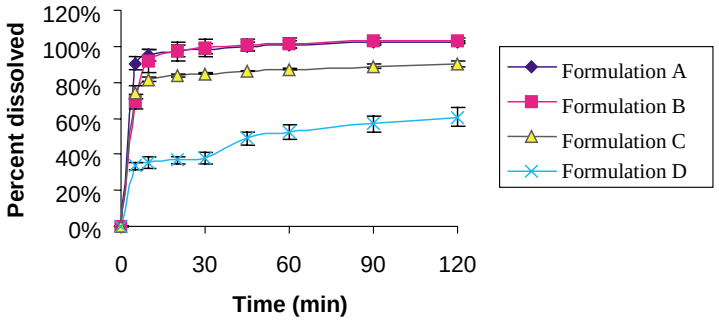

Fig. 2. Dissolution of various capsule formulations, each containing 187.5 mg of the mesylate salt of BMS-480188, in 1 l 0.01N HCl at 37 °C. The vertical bar represents the standard deviation ($n = 3$).

Table 2
Pharmacokinetics data of various formulations of the mesylate salt of BMS-480188

Formulation	C_{max} (ng/ml)	T_{max} (h)	AUC(0-T) (ng h/ml)	T_{half} (h)	Clearance (l/h/kg)	Vss (l/kg)	F (%)
A	13.58 (10.20)[a]	4.00 (1.00, 4.00)[b]	48.02 (17.67)[a]	–	–	–	5.7 (2.8)[a]
B	48.33 (7.76)[a]	1.00 (0.50, 4.00)[b]	194.35 (61.04)[a]	–	–	–	23.89 (11.2)[a]
Intravenous	3908.63 (1329.17)[a]	0.25 (0.25, 0.25)[b]	1757.51 (524.97)[a]	1.03 (0.15)[a]	2.99 (0.77)[a]	1.14 (0.23)[a]	–

[a] The standard deviations of the data are given in the parentheses.
[b] The minimum and maximum values of T_{max} are given in the parentheses.

dissolution medium similar to that in vivo (Kararli, 1995). The dissolution profiles of Formulations A–C using 30 ml medium are shown in Fig. 3. The amount of BMS-480188 dissolved from Formulation B is 80% greater than Formulation A. The dissolution of BMS-480188 is very slow in the pH range of 4–7. In 1 l of phosphate buffer at pH 7 at 37 °C, the solution concentration is 0.03 mg/ml after 3 h of dissolution of free base. Therefore, negligible amount of drug would dissolve in the intestine and the amount of absorbed drug will depend on the initial amount of drug dissolved in the stomach. The dissolution test in 30 ml medium shows that greater amount of drug is dissolved from Formulation B, which is consistent with the higher C_{max} and bioavailability in monkeys (Table 2).

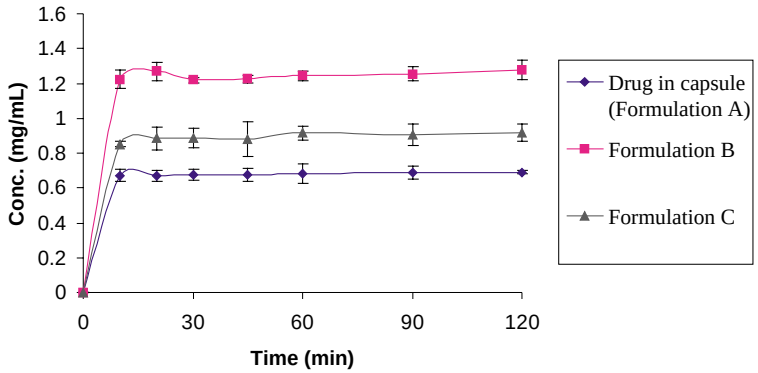

Fig. 3. Dissolution of capsule Formulations A–C, each containing 60 mg of the mesylate salt of BMS-480188, in 30 ml 0.01N HCl at 37 °C. The vertical bar represents the standard deviation ($n = 3$).

Although sodium lauryl sulfate (SLS) present in Formulation B is a wetting agent, the fast dissolution of the drug in capsule formulation (Formulation A) as shown in Figs. 2 and 3, indicates that BMS-480188 has no wetting issue. Therefore, the difference in the dissolution profile of Formulations A and B is unlikely due to the enhancement of wettability by SLS in Formulation B. The fast dissolution of Formulations A and B in 30 ml or 1 l dissolution medium also indicates that the difference in hydrodynamic conditions after change in the dissolution medium volume did not affect the initial dissolution of the formulation. The greater amount of drug dissolved from Formulation B in 30 ml dissolution medium can be explained by the following two possibilities: (1) the excipients increase the dissolution rate of the free base of BMS-480188 resulting from the conversion of the mesylate salt of BMS-480188 during dissolution test; (2) the excipients delay the conversion of the mesylate salt to the free base.

To test the first hypothesis, dissolution of capsule containing 150 mg free base (polymorph II) or a blend of 150 mg free base (polymorph II) + 150 mg excipients was compared in 1 l of 0.01N HCl containing 0.05 mg/ml BMS-480188 at 37 °C. The composition of excipients used is identical to that in Formulation B. The presence of BMS-480188 at 0.05 mg/ml in the initial dissolution medium enables the determination of the concentration at which the dissolution of free base ceases. The dissolution results are presented in Fig. 4. The dissolution profiles of pure free base and free base with excipients are identical, indicating that excipients do not affect the dissolution of free base. Therefore, the effect of excipients is likely related to the time of conversion of the mesylate salt to the free base but not on the dissolution of free base after conversion. For both systems, the dissolution almost ceases when the concentration of BMS-480188 in the dissolution medium reaches 0.09 mg/ml, suggesting that the dissolution of BMS-480188 in 0.01N HCl is negligible after the solution concentration is ≥0.09 mg/ml. The value of 0.09 mg/ml represents a pseudo-steady-state value under the experimental dissolution conditions, which is much lower than the equilibrium solubility of 1.08 mg/ml determined by vigorous shaking at 37 °C for 2 days. The slow dissolution of free base in 0.01N HCl is consistent with the concentration plateau observed in Fig. 3, as the dissolution ceased after the conversion of the salt to the free base.

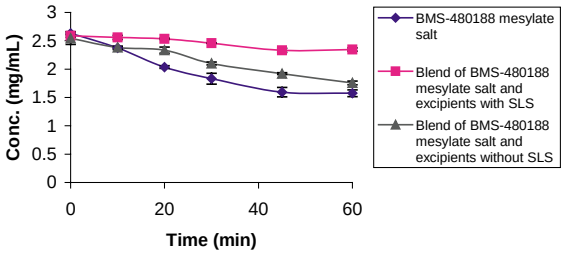

Fig. 5. Precipitation of BMS-480188 from 10 ml 0.01N HCl at 37 °C. The vertical bar represents the standard deviation ($n = 3$). The precipitated free base collected after 10 min is polymorph II.

Excipients are found to reduce the rate of solution mediated conversion of the salt to the free base. As shown in Fig. 5, excipients in Formulation B, particularly sodium lauryl sulfate, inhibit the precipitation of free base. During dissolution test using 30 ml medium, the concentration of the drug increases rapidly in the medium, which causes slow diffusion of the dissolved

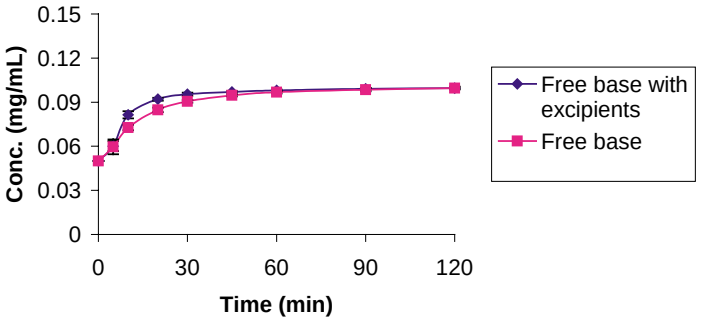

Fig. 4. Dissolution of capsules containing 150 mg BMS-480188 with/without excipients in 1 l 0.01N HCl with 0.05 mg/ml BMS-480188 at 37 °C. The vertical bar represents the standard deviation ($n = 3$).

drug from the dissolution interface to the bulk dissolution medium. This results in high concentration of the drug at the dissolution interface, causing the precipitation of the free base and subsequently inhibiting further dissolution. The concentration plateau in Fig. 3 suggests that dissolution ceases after the conversion of the salt to the free base. Therefore, the slower the conversion, the higher is the concentration of the dissolved drug. The presence of excipients inhibits the conversion to the free base during dissolution in 30 ml medium, which provides more time for the salt to dissolve, leading to greater amount of the dissolved drug. In the absence of excipients, the conversion to the free base may occur faster than in their presence. Therefore, during 30 ml dissolution test, greater amount of drug dissolved from Formulation B (drug with excipients) than from Formulation A (pure drug). As shown in Figs. 3 and 5, among the excipients, sodium lauryl sulfate plays the predominant role in inhibiting the conversion of the mesylate salt to the free base, and thereby leading to the greater amount of dissolved drug.

Assuming that the velocity of solution in a smaller volume stirred in a same speed is greater than that in a larger volume, the diffusion layer thickness in the large volume of dissolution medium will be greater than that in the small volume. However, in a large volume of dissolution medium, the sink condition is maintained and the concentration gradient from the dissolution interface to the bulk is much greater than that in a small volume of medium, which will lead to faster diffusion of the dissolved drug. The absence of precipitation during dissolution in 1 l medium indicates that the diffusion of the dissolved drug is fast such that the concentration of the drug in the dissolution interface is not high enough for the free base to precipitate. Under these conditions, the effect of excipients on the conversion of the free base is concealed. Because of the small volume of dissolution medium in vivo, precipitation of the free base is likely to occur in vivo. Therefore, in order to reveal the effect of excipients, it is necessary to conduct the dissolution test using the volume of dissolution medium similar to that in vivo.

It is also known that sodium lauryl sulfate as a surfactant in Formulation B may enhance the permeability of the drug, leading to improved bioavailability. However, according to the literature data, the improvement in the permeability caused by sodium lauryl sulfate is usually less than two-fold (Rege et al., 2001). Therefore, higher bioavailability of Formulation B is not likely to be caused by the effect of surfactant on permeability.

4. Conclusions

This study reports an interesting case of the importance of using physiologically relevant volume of dissolution medium in the in vitro dissolution test to predict the in vivo bioavailability. BMS-480188 mesylate, a salt of weak base, converts to free base through solution mediated transformation in the dissolution medium. The excipients can delay the conversion of the free base. Dissolution tests using large amount of dissolution medium under the sink condition does not reveal the conversion because of the fast diffusion of the solute at the dissolution interface. In this case, the excipients exert no effect on the dissolution profile. However, using smaller volume of dissolution medium (30 ml), the salt converts to the free base, causing decrease in the amount dissolved. The presence of excipients, particularly sodium lauryl sulfate, slows the conversion of the salt to the free base thereby the amount of drug dissolved is greater from the formulated capsule than the capsule containing the drug alone. These results explain the higher bioavailability of the formulated capsule. Because the volume of gastric fluid in the fasted state is very small, a dissolution test with the ratio of dose to volume of dissolution medium similar to that in vivo may provide a better prediction of the in vivo dissolution profile of a formulation. This is applicable to drugs, which are salts of weak base with very low intrinsic solubility. It should be noted that while dissolution tests using physiologically relevant volumes, as demonstrated here, can be a useful tool to screen prototype formulations during drug development, such tests (which often involve non-sink conditions) may not be appropriate as a quality control tool for the testing and release of established solid dosage formulations.

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