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Unit Dose Sampling: A Tale of Two Thieves

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ABSTRACT

As a consequence of the United States vs. Barr Laboratories decision, pharmaceutical companies are now compelled to demonstrate the uniformity of unit dose samples of final powder blends. This investigation was initiated in response to a previously reported failed attempt to validate a process for lower strengths of a currently marketed product. This process has a long history of providing high-quality commercial tablets at the higher strength. The failure occurred because the unit dose samples of the final powder blend were indisputably subpotent. Interestingly, during the validation effort, 799 tablets of various strengths were assayed and exhibited outstanding content uniformity and potency. It was hypothesized that this failure was due to sampling bias which occurred when small (unit dose) samples were extracted with a thief from a static powder bed that was 7 orders of magnitude greater in size. The purpose of this two-part investigation was to test this hypothesis. In both of these studies, sieve analyses were conducted on samples collected from 100 kg of the final commercial blend. From these data the ratio of the coarse to fine fractions was calculated. This ratio is directly proportional to particle size and, for this product, related to the concentration of drug in the sample. Two different thieves (A and B) and a variety of sampling conditions were compared in Study 1 and the results suggest that the coarse-to-fine ratio: (a) decreases with sampling depth, (b) is generally larger for samples extracted with thief B than thief A, and (c) is larger for samples collected with a thief that is maintained in the vertical position than one held at an acute angle. The intent of Study 2, which focused exclusively on thief B, was to determine if the pronounced effect of sampling depth on the coarse-to-fine ratio was due to sampling bias or product segregation. The results of Study 2 demonstrated that coarser material was preferentially sampled from the top of the bed than from the bottom. These studies indicated that one thief used under different conditions and two thieves used

under similar conditions can extract samples of different particle size from the same population. This implicates sampling bias and confirms that a thief is far from an ideal sampling device. We believe that the unit dose sampling requirement is more likely to impede the delivery of innovative pharmaceutical products to the marketplace than it is to enhance the quality of these products.

INTRODUCTION

The manufacturers of pharmaceutical products are required to validate their manufacturing processes. A critical component of process validation, for a solid dosage form, consists of demonstrating the uniformity of the final powder blend as well as the drug product. Until recently, pharmaceutical companies were given a relatively free hand in developing validation programs and standards. This created inconsistencies in practices throughout the industry. The situation changed dramatically in 1993 as a result of a pivotal court case, the *United States vs. Barr Laboratories* (1). In his landmark ruling, Judge Alfred M. Wolin defined some of the CGMP requirements for process validation of solid dosage forms in greater detail than is specified in 21, Code of Federal Regulations, Part 211. As part of his decision the judge determined that the appropriate sample size for content uniformity testing of final powder blends is at most three times the run weight of the finished product. This is now commonly referred to as the unit dose sampling requirement. Although Judge Wolin did not specify the criteria that should be used to evaluate the uniformity of final blends, the Food and Drug Administration (FDA) has sometimes suggested (2) that they should be held to a higher standard than that of the finished product.

Overall, the Wolin decision has had a profound and generally positive impact on the pharmaceutical industry. Since this precedent setting ruling many pharmaceutical companies have critically reviewed their process validation procedures. The unit dose sampling requirement, however, is an onerous consequence of the Wolin decision. Conceptually, sampling unit doses of powder from a blender or hopper to demonstrate content uniformity seems logical. But in practice many problems can occur when small samples are collected from a static powder bed that is over 7 orders of magnitude greater in size (3–6), as is often required during the validation of a commercial product. This problem is exacerbated in the pharmaceutical industry where these samples must then be held to very high standards.

A previous communication (7) described an attempt to validate the manufacturing process for a tablet that contained either X mg or $2X$ mg of drug substance (DS). The final dosage forms were produced by compressing the final powder blend used in a marketed product to proportionately smaller running weights. The process used to manufacture the original product was validated and had a long history of providing tablets that exhibited consistent and excellent uniformity of DS at $4X$ mg per dosage unit. As specified in the Wolin decision the revised validation program for the new lower-strength tablets was designed to demonstrate content uniformity of the final blend, both in the V blender and after discharge into hoppers, at weights that corresponded to X mg of DS per sample. Obviously, uniformity of the blend at X mg of DS ensures uniformity at all higher strengths. As part of that program, unit dose samples were extracted from a V blender and discharge hoppers using a sample thief that was constructed by Hoechst Marion Roussel's (formerly Marion Merrell Dow) machine shop.

Three successive full-scale batches of lubricated granulation were manufactured, sampled, and analyzed, at each of two manufacturing sites, according to an approved validation protocol. Each batch was compressed into approximately 1 million X mg tablets and a half a million $2X$ mg tablets; the remaining granulation was compressed into salable product at $4X$ mg of DS per unit. The acceptance criteria for the unit dose samples of the final blend were based on the USP Uniformity Criteria for finished tablets (i.e., individual units between 85% and 115% of label claim with an RSD less than or equal to 6%).

As part of this multisite validation effort, and subsequent investigation, 799 tablets of various strengths were assayed for content uniformity. The tablets from each of the validation batches readily passed the acceptance criteria specified in the validation protocol. The mean potency of these tablets was marginally greater than 100% of label claim. Interestingly, extensive content uniformity data from each of the six validation batches demonstrated consistently and significantly lower drug content in the unit dose samples than in the tablets. In fact, out of 504 powder samples analyzed, only 36 (7.1%) contained DS levels at or above 100% of label claim; of these, only 7 (1.4%) contained more than

103% of the theoretical quantity. In other words, the unit dose data suggested that the powder blend was subpotent and derived from a different population than the tablets. The variability of the drug content of the powder samples, as characterized by the standard deviation was, for the most part, only slightly greater than that of the tablets. Unfortunately, the validation effort for the new lower-strength products was unsuccessful because the unit dose samples from some of the batches did not conform to the acceptance criteria. The investigators hypothesized that the unreasonably low potency level of the lubricated granulation, and the failure of the validation effort, was due to sampling bias and inconsistent sampling technique. The purpose of this investigation, which was divided into two parts, Study 1 and Study 2, was to test this hypothesis.

METHODS: STUDY 1

Demonstrating sampling bias is a difficult and labor-intensive task. Our approach was to collect numerous unit dose samples, under a variety of controlled conditions, using two different sampling thieves. Sieve analyses were then performed for 5 min on entire samples using an ATM Model L3 Sonic Sifter (Allen Bradley Co., Milwaukee, WI) with pulse and sift settings of 3. The net weights of the 20, 40, 70, 140, 200, and fine mesh cuts were recorded for each sample. From these data, the ratios of the coarse to fine mesh fractions were calculated. The coarse-to-fine ratio is defined as the sum of the 20, 40, and 70 mesh cut net sample weights divided by the sum of the 140, 200, and fine mesh cut net sample weights. This ratio is directly proportional to particle size and, as explained below, is also related to the concentration of DS in the powder samples.

The material used in this study was granulated and blended at commercial scale. In this process, the drug is first wet granulated with excipients and the resulting granules are then blended with additional excipients in a V blender. This material is then discharged into hoppers and eventually compressed into tablets using a high-speed rotary tablet press. This formulation contains a relatively high proportion of fine intra- and extragranular excipients. In addition, the small quantity of DS resides exclusively in the relatively coarse granules. Thus, it is not surprising that assays of individual mesh cuts of the final blend indicate that the concentration of DS is proportional to the coarse-to-fine ratio.

Approximately 100 kg of the final powder blend was obtained during discharge from the V blender; the remainder of the blend was compressed into commercial tablets at $4X$ mg of DS per dosage unit. This material was manufactured in our commercial manufacturing location (site A in Ref. 7). The blend was delivered to our R&D facility in double-lined polyethylene bags inside a fiber drum that was approximately 75 cm tall with a capacity of around 150 liters. The blend was loaded into a Patterson–Kelly 5 ft^3 Twin Shell (V) Blender and mixed for two full revolutions at 24 rpm. The material from the V blender was then discharged back into double polyethylene bags that lined the fiber drum. After discharge, the height of the material in the drum was approximately 56 cm.

As part of this study, two different sample thieves were used to extract targeted unit dose samples from the drum in a controlled manner. The first thief, which was constructed by Hoechst Marion Roussel's machine shop, is described in Ref. 7 and is identified as thief A in this communication. The second thief, a GlobePharma (Englewood, CA) Model IV equipped with two 0.3-cc sample chambers is referred to as thief B. Although both of these thieves consist of two concentric tubes their geometries are distinctly different. In addition, the sampling chambers of thief A were constructed of Teflon™ whereas those of thief B were made of stainless steel. Thief A was designed to remove triplicate samples that contain X mg of DS and triplicate samples that contain $2X$ mg of DS per stab. These six sampling chambers are aligned vertically near the bottom of the thief. In this study, only two targeted unit dose samples were collected per stab. Thief B, on the other hand, was designed to remove only two unit dose samples per stab. The two sampling chambers are also aligned vertically near the bottom of the thief.

Both of these thieves were operated in a similar manner. After they were inserted into the static powder bed their inner tube was rotated to cover the sampling chambers. Ideally, upon removal from the bed, any material caught in the space between the inner tube and the opening in the outer tube should be discarded in order to capture only the material inside the sample chambers. To simulate proper and careless sampling practices, two different techniques were used to collect the two powder samples from each stab of both thieves. In technique I, only the powder within the sample chamber was collected; the material caught between the inner tube and outer tube of the thief was discarded. In technique II, the material in the sample chamber and the material caught between the inner tube and outer tube was collected. Thus, for each stab of thief A, one A-I and one A-II sample was collected. Similarly, for each stab of thief B, one B-I and one B-II sample was col-

lected. These thief/technique sampling scenarios are summarized in Table 1. Due to differences in the design of the two thieves, the technique I sample was collected from the bottom chamber of thief A and the top chamber of thief B whereas the technique II sample was collected from the next-to-bottom chamber of thief A and the bottom chamber of thief B.

In theory, sampling depth can have a considerable impact on the quality of the sample. It is the static pressure in a powder bed that forces the bulk powder to flow into the sample chamber of a thief. Since the static pressure is greater at the bottom of a bed than in the middle or near the top, it is possible that the depth of penetration of the thief can influence the distribution of particles that flow into the sample chambers. Inserting a thief into a static bed also forces material from the upper layers downward; this is called channeling. Channeling disturbs the bed and can lead to sampling bias. Furthermore, the force necessary to insert a thief into a powder bed can be appreciable and is proportional to the depth of penetration. This can lead to particle attrition and additional sampling bias. In order to determine the effect of these factors, samples were collected from two depths within the powder bed: at approximately 10 cm (at a height of approximately 46 cm) and 46 cm (at a height of approximately 10 cm) below the surface.

Thieves are intended to be used in a vertical position. This can be difficult or impossible when sampling from a V blender. If a thief is not used in a perfectly vertical position, the sample chamber openings can be oriented downwards (6:00), upwards (12:00), or somewhere in between during sampling; refer to Fig. 1. In order to study the influence of the orientation of the sample chambers, samples were collected with the thieves maintained at 90° (i.e., vertical) and 45° angles with the horizontal. Samples were then collected with the sample chambers oriented at 6:00, 3:00, or 12:00, at both the top and bottom of the bed, for each of the four thief/technique scenarios presented in Table 1. Obviously, the orientation of the sample chambers is unique when the thief is used in the vertical position.

This study was designed to investigate the effect of three sampling factors: (*a*) thief/technique (four condi-

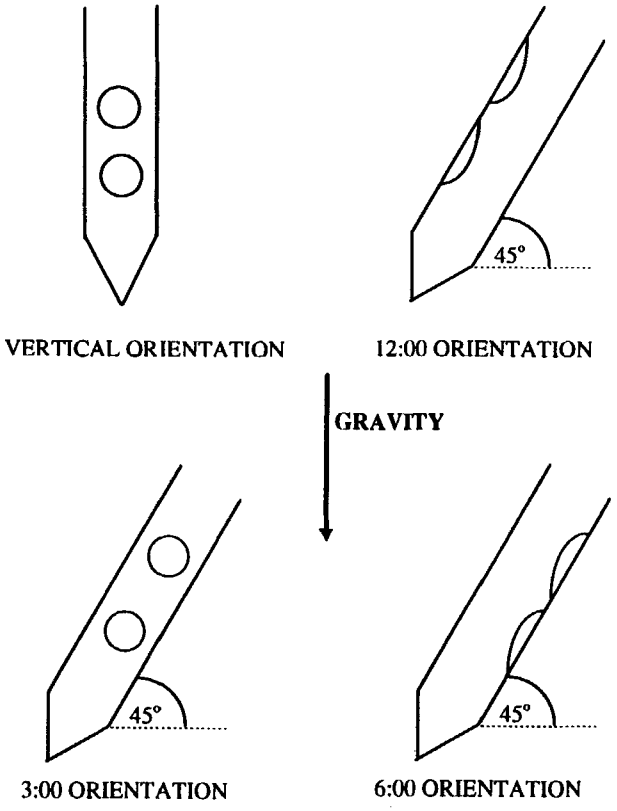

Figure 1. Orientation of sample chambers (not drawn to scale).

Table 1

Thief/Technique Sampling Scenarios

Code	Thief	Technique
A-I	Hoechst Marion Roussel (7)	Sample chamber only
A-II	Hoechst Marion Roussel (7)	Sample chamber plus material caught between inner tube and outer tube
B-I	GlobePharma Model IV (0.3-cc chamber)	Sample chamber only
B-II	GlobePharma Model IV (0.3-cc chamber)	Sample chamber plus material caught between inner tube and outer tube

tions), (*b*) sample depth (two conditions), and (*c*) orientation of the sample chambers (four conditions) on the coarse-to-fine ratio of the unit dose samples. These factors were studied in a factorial manner. Triplicate samples (i.e., three thief stabs) were collected from the static powder bed according to the sampling protocol outlined below. Special care was taken not to stab the same area of the bed twice.

1. Vertical samples were collected from approximately 10 cm below the surface of the bed using each thief. This resulted in 6 thief stabs (12 samples) including replicates. The sampling order was randomized.
2. The drum was placed on a hand truck which was tilted until it was at an angle of approximately 45° with the floor. The thieves were inserted at the 6:00 position *of the drum*, and parallel to its wall, just below the surface of the bed, with the sample chambers oriented at 6:00, 3:00, or 12:00. The drum was slightly rotated after each thief stab so that subsequent stabs would always be made into undisturbed powder at the 6:00 position *of the drum*. This resulted in 18 thief stabs (36 samples) including replicates. The sampling order was randomized.
3. The drum was returned to the vertical position and vertical samples were collected from the bottom of the bed at a depth of approximately 46 cm below the surface. This resulted in 6 thief stabs (12 samples) including replicates. The sampling order was randomized.
4. The hand truck was tilted until it was at an angle of approximately 45° with the floor. The thieves were inserted at the 6:00 position *of the drum*, and parallel to its wall, into the bottom of the bed, with the sample chambers oriented at 6:00, 3:00, or 12:00. The drum was slightly rotated after each thief stab so that subsequent stabs would always be made into undisturbed powder at the 6:00 position *of the drum*. This resulted in 18 thief stabs (36 samples) including replicates. The sampling order was randomized.

Each sample was collected in a pouch made of glassine paper. A visual inspection of the sample chambers suggested that the formulation did not adhere to either the Teflon (in the case of thief A) or the stainless steel (in the case of thief B) cavities. A sieve analysis was conducted on two of the replicates. The third replicate was stored for possible further analysis.

RESULTS AND DISCUSSION: STUDY I

An analysis of variance in three factors (thief/technique, sample depth, and orientation of the sample chambers) was conducted on the data from Study 1. The data are listed in Appendix A-1. The three-factor analysis of variance table is given in Appendix A-2. Data from the 6:00 chamber orientation (refer to Fig. 1) were not included in this analysis since a sufficient quantity of sample was not retained in the cavity at this orientation. With the exception of the [B-1, bottom, 3:00] sampling condition, where all but one replicate sample were lost to spillage during handling, the analysis was performed using the coarse-to-fine ratio data from each of two replicate samples. Because of the unequal number of replicates in the data (one replicate for sample [B-I, bottom, 3:00] and two replicates for all other conditions), it was necessary to conduct the analysis of variance using SAS® (8) Proc GLM Type III sums of squares and to report the least squares means rather than the raw data means.

Due to the significant depth × thief/technique ($p = 0.0224$) and depth × orientation ($p = 0.0030$) interactions (refer to Appendix A-2) and due to the much larger standard deviation ($\sqrt{\text{MSE}}$) for the top samples compared to the bottom samples, a separate analysis of variance was conducted for each depth (top and bottom). These two-factor (thief/technique and orientation) analyses of variance tables are given in Appendixes A-3 and A-4.

The least squares factor means are given in Table 2. In this table, for a given factor, means flagged with the same letter do not differ significantly at the $\alpha = 0.10$ level. Least squares cell means (for each thief/technique and orientation combination) are given for each depth (top and bottom) in Tables 3 and 4. Table 3 provides a comparison of thief/technique means for each depth and orientation combination. Means flagged by the same letter within a depth and orientation combination do not differ at the $\alpha = 0.10$ level. Table 4 provides a comparison of orientation means for each depth and thief/technique combination. Means flagged by the same letter within a depth and thief/technique combination do not differ at the $\alpha = 0.10$ level.

The least squares means comparison (refer to Table 2) indicates that sample depth had a significant effect on the coarse-to-fine ratio. Thief samples taken near the top of the bed had a higher coarse-to-fine ratio than samples taken from the bottom of the bed. This effect was consistent across all combinations of thief/technique and

Table 2

Least Squares Factor Means for Study 1

Factor	Level	Mean Coarse: Fine Ratio
Depth	Top	2.46 C[a]
	Bottom	1.07 D
Thief/Technique	A-I	1.27 C
	A-II	1.27 C
	B-I	2.42 D
	B-II	2.09 D
Orientation	Vertical	2.47 C
	12:00	1.37 D
	3:00	1.46 D

[a]Letters indicate the results of a 2-sided *t* test at $\alpha = 0.1$. For a given factor, means that are flagged with the same letter do not differ significantly at $\alpha = 0.1$.

sample chamber orientation (refer to Table 3 or Table 4). The mean coarse-to-fine ratios were 2.46 and 1.07 for top and bottom samples, respectively. This statistically significant result is also of practical significance. In all probability, the unit dose samples of this product that were collected from the bottom of the drum had a lower potency than those collected from the top of the drum.

It is apparent in Tables 2 and 3 that the type of thief, but not the sampling technique, had a significant effect on the coarse-to-fine ratio. Thief B tended to produce samples with a higher coarse-to-fine ratio than thief A. This effect was generally consistent across all combinations of sample depth and sample chamber orientation. This thief effect suggests that at least one of these thieves provides a biased sample. In fact, if thief B rather than thief A had been used in the previously reported validation attempt (7), it is possible that the potency of the unit dose samples would have been a little higher and more representative of the tablets. In other words, we may have been robbed by our own thief!

In Tables 2 and 4 it is apparent that the orientation of the sample chambers also had a significant effect on the coarse-to-fine ratio. The vertical thief position produced the highest coarse-to-fine ratio, regardless of the sample depth and thief/technique.

The orientation of thief A was not controlled or recorded during the previous validation effort (7). The results presented in Table 4, however, suggest that the orientation of the sample chamber of thief A has little effect on the coarse-to-fine ratio when the thief is main-

Table 3

Least Squares Means for Comparing Thief/Technique at Each Combination of Depth and Orientation in Study 1

Orientation	Thief/Technique	Mean Coarse: Fine ratio Top	Bottom
Vertical	A-I	1.97 C[a]	1.00 C
	A-II	2.22 C	0.71 D
	B-I	6.23 D	1.95 E
	B-II	3.86 E	1.82 E
12:00	A-I	1.45 C	0.99 C
	A-II	1.48 C	0.90 C
	B-I	1.55 C	1.05 C
	B-II	1.93 C	1.57 D
3:00	A-I	1.39 C	0.83 C
	A-II	1.70 CD	0.59 D
	B-I	3.13 D	0.63 CD
	B-II	2.58 CD	0.80 CD

[a]Letters indicate the results of a 2-sided *t* test at $\alpha = 0.1$. For a given depth and orientation, means with the same letter do not differ significantly at $\alpha = 0.1$.

Table 4

Least Squares Means for Comparing Orientation at Each Combination of Depth and Thief/Technique in Study 1

Thief/Technique	Orientation	Mean Coarse: Fine Ratio Top	Bottom
A-I	Vertical	1.97 C[a]	1.00 C
	12:00	1.45 C	0.99 C
	3:00	1.39 C	0.83 C
A-II	Vertical	2.22 C	0.71 CD
	12:00	1.48 C	0.90 C
	3:00	1.70 C	0.59 D
B-I	Vertical	6.23 C	1.95 C
	12:00	1.55 D	1.05 D
	3:00	3.13 E	0.63 E
B-II	Vertical	3.86 C	1.82 C
	12:00	1.93 D	1.57 D
	3:00	2.58 CD	0.80 E

[a]Letters indicate the results of a 2-sided *t* test at $\alpha = 0.1$. For a given depth and thief/technique, means with the same letter do not differ significantly at $\alpha = 0.1$.

tained at a 45° angle. The results for thief B, however, are more dependent on sample chamber orientation.

METHODS: STUDY 2

The most significant finding of Study 1 was that sampling depth has a profound impact on the coarse-to-fine ratio of the sample. These data suggest that this ratio and the potency of the unit dose samples decrease with depth. We designed and executed a second study, Study 2, in order to determine if this effect was due to sampling bias or actual segregation of the powder.

The approximately 100 kg of material that was used in Study 1 was returned to the 5-ft^3 Patterson–Kelly Twin Shell Blender and mixed for two revolutions. The powder was then discharged back into the fiber drum, which was lined with two polyethylene bags. After discharge, the height of the material in the drum was approximately 56 cm.

The sampling protocol for Study 2 is shown schematically in Fig. 2 and described below. Six replicate samples were collected with the bottom chamber of thief B, in the vertical position, using technique I (refer to Table 1). (Note: In Study 1 the B-I samples were collected with the top chamber of thief B). The primary response variable was the coarse-to-fine ratio.

1. *Full Drum*: The height of the powder bed was approximately 56 cm. A sampling cup was used to collect two 10-g samples from the surface of the powder bed. Six unit dose thief samples were collected at a depth of approximately 10 cm at equidistant locations around an imaginary circle with the center coincident with the drum center and the radius equal to approximately half the drum radius (i.e., approximately half way between the drum center and the drum wall). Six thief samples were then collected around the imaginary circle at a depth of approximately 28 cm (i.e., near the middle of the bed). Finally, six thief samples were collected around the imaginary circle at a depth of approximately 46 cm (i.e., near the bottom of the bed).
2. *Partially Full Drum*: The material in the top of the drum was carefully and gently removed using a scoop until the powder bed was approximately 38 cm in height. Two 10-g samples were collected from the top of the bed using a sampling cup. Six thief samples were then collected around the imaginary circle at a depth of approximately 10 cm (just below the surface of the bed).
3. *Almost Empty Drum*: Additional material was carefully and gently removed using a scoop until the powder bed was approximately 20 cm in height. Two 10-g samples were collected from the top of the bed using a sampling cup. Six thief samples were then collected around the imaginary circle at a depth of approximately 10 cm (i.e., just below the surface and, now, near the bottom of the bed).

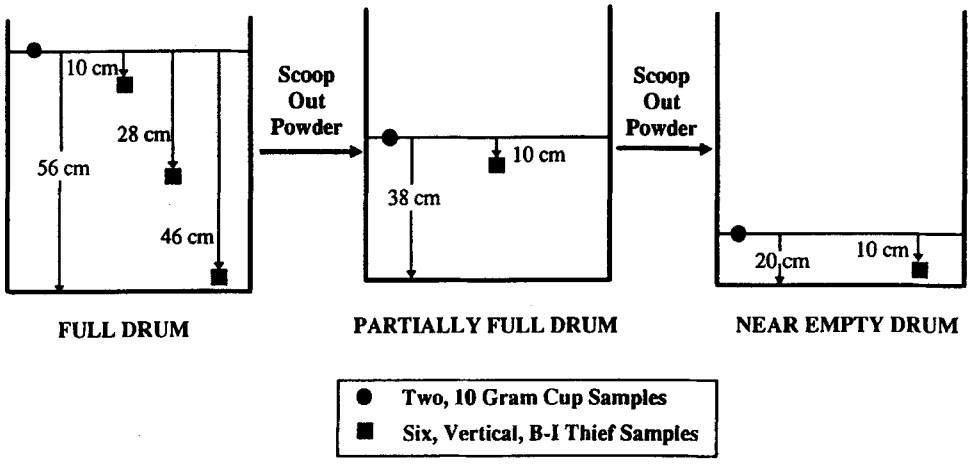

Figure 2. Sampling plan for Study 2 (not drawn to scale).

Each sample was collected in a pouch made of glassine paper. Particle size measurements were conducted on all of the samples.

RESULTS AND DISCUSSION: STUDY 2

Study 2 was designed to investigate the effect of one factor (drum fill level) on the coarse-to-fine ratio of surface cup samples and of two factors (drum fill level and sample depth) on thief samples. This study allowed us to compare the course-to-fine ratios of thief samples collected from the same height in the bed, but at different depths, to surface cup samples collected at the same height. It was assumed that the larger cup samples were more representative of the powder population than the smaller thief samples (6).

A single-factor analysis of variance was conducted on the data from Study 2. The data are listed in Appendix B-1. The eight levels of the single factor analysis of variance corresponded to the three cup sample locations and the five thief sample locations (refer to Fig. 2). The analysis of variance (refer to Appendix B-2) indicated that the mean coarse-to-fine ratio differed significantly ($p = 0.0001$) among the eight sampling conditions. This significant result was followed by performing pairwise two-sided t tests ($\alpha = 0.10$) to compare the mean coarse-to-fine ratio among the three cup sample locations, among the five thief sample locations, and to compare each cup sample location to its corresponding thief sample location.

A summary of the data from this study is presented in Fig. 3. This figure portrays the mean coarse-to-fine ratios of the cup and thief samples as a function of drum fill level and, in the case of the thief samples, as a function of sample depth. For the cup samples, which were always collected from the surface of the bed, this ratio was significantly lower when the drum was full (1.4) than when it was partially full (1.7). This difference, however, may not be of practical significance.

For the thief samples, the mean coarse-to-fine ratio differed significantly among the three sample depths when the drum was full. These means were 2.0 at a depth of 10 cm below the surface, 1.7 at a depth of 28 cm, and 1.5 at a depth of 46 cm. When the drum was partially full the mean coarse-to-fine ratio was significantly higher (2.0) than when the same location (i.e., height measured from the bottom of the drum) was sampled from a full drum (1.7). When the drum was near empty, the mean ratio (2.3) was also significantly higher than when this location was sampled from a full drum (1.5). Some of these statistical differences are also of practical significance.

In the second part of the analysis, cup samples were compared to thief samples that were collected at the same height in the bed; refer to Fig. 3. For all levels of drum fill, the thief samples that were collected just below the surface of the bed had statistically higher mean coarse-to-fine ratios than the cup samples that were collected at the surface. There was, however, no statistical difference in this ratio when the thief samples

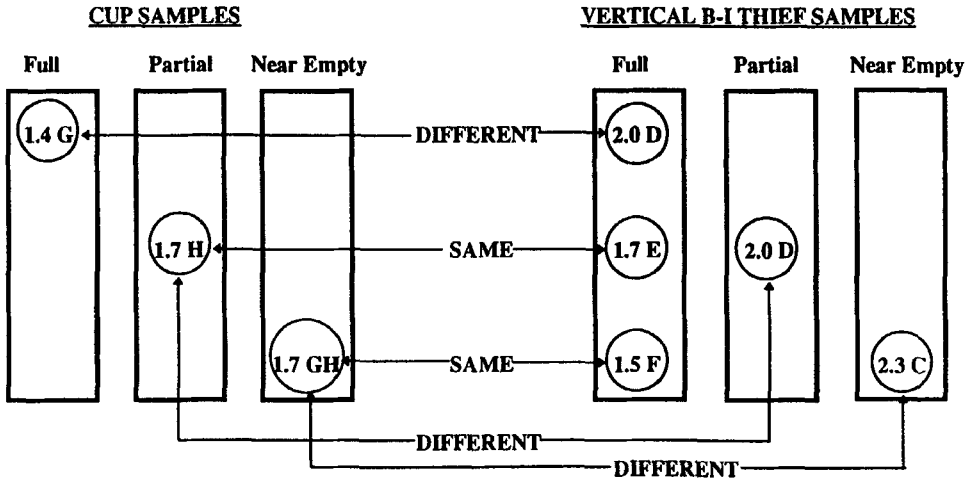

Figure 3. Results of sampling Study 2. Numbers represent the mean coarse-to-fine ratio for cup samples ($n = 2$) or thief samples ($n = 6$). Within a sample type (cup or thief), means flagged by the same letter do not differ significantly based on 2-sided t tests at $\alpha = 0.1$. Results of 2-sided t tests ($\alpha = 0.1$) for comparing cup means to thief means are indicated by arrows.

collected at the middle and bottom of a full drum were compared to the cup samples collected at the same height from the surface of a partially full and near empty drum, respectively.

These data confirm the results observed in Study 1 that the coarse-to-fine ratio, for this product, decreases with sampling depth. The results of this study provide additional clarity of this phenomenon. Rather than preferentially extracting finer material at greater sampling depths, as was originally anticipated, this thief appears to preferentially extract coarser material at smaller depths.

CONCLUSIONS

The purpose of this investigation was to determine if sampling bias can occur when small samples are extracted from a larger static powder bed with a thief. A secondary purpose was to study the influence of sampling technique on potential bias. This investigation was divided into two separate studies which were conducted on the same population of powder.

The intent of the first study was to quantify the effect of thief/technique, sample depth, and sample chamber orientation on the coarse-to-fine ratio of unit dose samples. This ratio is directly proportional to the particle size of the sample and, for this product, related to drug content. The results of Study 1 suggest that the coarse to fine ratio: (*a*) decreases with sample depth, (*b*) is generally larger for samples extracted with thief B than thief A, and (*c*) is larger for samples collected with a vertical thief than one maintained at an acute angle.

The intent of the second study, which focused exclusively on thief B, was to determine if the effect of sampling depth on particle size was due to sampling bias or segregation of the powder. The results of Study 2 indicate that thief B preferentially extracts coarser material from the top of the static bed than from the bottom. Unfortunately, we were not equipped to study this effect at depths characteristic of a commercial-scale process.

We have demonstrated that one thief used under different conditions and two thieves used under similar conditions can extract samples of different particle size from the same population. These results implicate sampling bias. Since each formulation and process is different, care must be taken not to assume that these results apply to other products and/or thieves. Together, the results of these studies suggest, as others have indicated (3–7), that a thief is far from the ideal sampling device.

It is reasonable to expect pharmaceutical companies to demonstrate the uniformity of the final blends of their solid products. It is unreasonable, however, to force them to rely on unit dose sampling to accomplish this goal. Our failed process validation program and subsequent investigations, including these studies, consumed an incredible amount of time, money, and resources. The focus of this aspect of process validation should be to demonstrate uniformity of the final powder blend, not sampling bias. Sampling bias can readily occur when small samples are extracted from large static powder beds. Pharmaceutical manufacturers must be allowed to take larger samples during process validation or the flow of new drug products to the marketplace will be severely and unnecessarily impeded.

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APPENDIX A-1: DATA LISTING FOR STUDY 1

Sample No.	Stab	Depth	Thief/Technique	Orientation	Replicate	Coarse:Fine
1	1	Top	A-I	Vertical	1	1.96
2	1	Top	A-II	Vertical	1	2.03
3	2	Top	B-I	Vertical	1	7.46
4	2	Top	B-II	Vertical	1	2.93
5	3	Top	A-I	Vertical	2	1.98
6	3	Top	A-II	Vertical	2	2.40
7	4	Top	B-I	Vertical	2	4.99
8	4	Top	B-II	Vertical	2	4.79
9	5	Top	A-I	12:00	1	1.44
10	5	Top	A-II	12:00	1	1.54
11	6	Top	B-I	12:00	1	1.77
12	6	Top	B-II	12:00	1	1.93
13	7	Top	A-I	3:00	1	1.51
14	7	Top	A-II	3:00	1	1.59
15	8	Top	B-I	3:00	1	2.04
16	8	Top	B-II	3:00	1	1.69
17	9	Top	A-I	12:00	2	1.46
18	9	Top	A-II	12:00	2	1.43
19	10	Top	B-I	12:00	2	1.33
20	10	Top	B-II	12:00	2	1.93
21	11	Top	A-I	3:00	2	1.28
22	11	Top	A-II	3:00	2	1.81
23	12	Top	B-I	3:00	2	4.22
24	12	Top	B-II	3:00	2	3.48
25	13	Bottom	A-I	Vertical	1	1.01
26	13	Bottom	A-II	Vertical	1	0.66
27	14	Bottom	B-I	Vertical	1	1.85
28	14	Bottom	B-II	Vertical	1	1.62
29	15	Bottom	A-I	Vertical	2	1.00
30	15	Bottom	A-II	Vertical	2	0.76
31	16	Bottom	B-I	Vertical	2	2.06
32	16	Bottom	B-II	Vertical	2	2.03
33	17	Bottom	A-I	12:00	1	1.01
34	17	Bottom	A-II	12:00	1	0.91
35	18	Bottom	B-I	12:00	1	1.05
36	18	Bottom	B-II	12:00	1	1.60
37	19	Bottom	A-I	3:00	1	0.85
38	19	Bottom	A-II	3:00	1	0.67
39	20	Bottom	B-I	3:00	1	
40	20	Bottom	B-II	3:00	1	0.68
41	21	Bottom	A-I	12:00	2	0.98
42	21	Bottom	A-II	12:00	2	0.89
43	22	Bottom	B-I	12:00	2	1.05
44	22	Bottom	B-II	12:00	2	1.54
45	23	Bottom	A-I	3:00	2	0.80
46	23	Bottom	A-II	3:00	2	0.51
47	24	Bottom	B-I	3:00	2	0.63
48	24	Bottom	B-II	3:00	2	0.92

APPENDIX A-2: THREE-FACTOR ANALYSIS OF VARIANCE FOR COARSE:FINE RATIO: STUDY 1

Source of Variability	Degrees of Freedom	Type III Sum of Squares[a]	Mean Square	*F* Value	Prob. > *F* (*p* Value)
Depth	1	22.12	22.12	55.68	0.0001
Thief/technique	3	11.62	3.87	9.75	0.0002
Orientation	2	11.85	5.92	14.91	0.0001
Depth × Thief/technique	3	4.61	1.54	3.87	0.0224
Depth × orientation	2	5.98	2.99	7.53	0.0030
Thief/technique × orientation	6	9.09	1.52	3.82	0.0087
Depth × thief/technique × orientation	6	3.35	0.56	1.40	0.2556
Error	23	9.14	0.40		
Total (corrected)	46	78.91			

Note. Overall mean (n = 47) = 1.788; $\sqrt{MSE}$ = 0.6303; CV = 35.25%.
[a]Computed using SAS® Proc GLM.

APPENDIX A-3: TWO-FACTOR ANALYSIS OF VARIANCE FOR COARSE:FINE RATIO TOP SAMPLES: STUDY 1

Source of Variability	Degrees of Freedom	Type III Sum of Squares[a]	Mean Square	*F* Value	Prob. > *F* (*p* Value)
Thief/technique	3	15.93	5.31	7.10	0.0053
Orientation	2	16.24	8.12	10.85	0.0020
Thief/technique × orientation	6	11.22	1.87	2.50	0.0834
Error	12	8.98	0.75		
Total (corrected)	23	52.37			

Note. Overall mean (n = 24) = 2.457; $\sqrt{MSE}$ = 0.8651; CV = 35.21%.
[a]Computed using SAS® Proc GLM.

APPENDIX A-4: TWO-FACTOR ANALYSIS OF VARIANCE FOR COARSE:FINE RATIO BOTTOM SAMPLES: STUDY 1

Source of Variability	Degrees of Freedom	Type III Sum of Squares[a]	Mean Square	*F* Value	Prob. > *F* (*p* Value)
Thief/technique	3	1.52	0.51	35.26	0.0001
Orientation	2	1.55	0.78	54.11	0.0001
Thief/technique × orientation	6	1.24	0.21	14.44	0.0001
Error	11	0.16	0.01		
Total (corrected)	22	4.59			

Note. Overall mean (n = 23) = 1.090; $\sqrt{MSE}$ = 0.1199; CV = 10.99%.
[a]Computed using SAS® Proc GLM.

APPENDIX B-1: DATA LISTING FOR STUDY 2

Sample Number	Sample Type	Drum Fill Level (cm)	Sample Depth from Surface (cm)	Coarse:Fine Ratio
1	Cup	56	—	1.47
2	Cup	56	—	1.34
3	Thief	56	10	1.78
4	Thief	56	10	2.15
5	Thief	56	10	2.14
6	Thief	56	10	1.92
7	Thief	56	10	1.95
8	Thief	56	10	1.90
9	Thief	56	28	1.89
10	Thief	56	28	1.74
11	Thief	56	28	1.66
12	Thief	56	28	1.73
13	Thief	56	28	1.45
14	Thief	56	28	1.88
15	Thief	56	46	1.69
16	Thief	56	46	1.72
17	Thief	56	46	1.36
18	Thief	56	46	1.50
19	Thief	56	46	1.48
20	Thief	56	46	1.40
21	Cup	38	—	1.73
22	Cup	38	—	1.74
23	Thief	38	10	2.17
24	Thief	38	10	2.27
25	Thief	38	10	2.14
26	Thief	38	10	1.87
27	Thief	38	10	1.54
28	Thief	38	10	2.26
29	Cup	20	—	1.59
30	Cup	20	—	1.81
31	Thief	20	10	2.47
32	Thief	20	10	2.52
33	Thief	20	10	2.27
34	Thief	20	10	2.19
35	Thief	20	10	2.28
36	Thief	20	10	2.12

APPENDIX B-2: SINGLE-FACTOR ANALYSIS OF VARIANCE FOR COARSE:FINE RATIO: STUDY 2

Source of Variability	Degrees of Freedom	Type III Sum of Squares[a]	Mean Square	*F* Value	Prob. > *F* (*p*-Value)
Sample type/location	7	2.76	0.39	12.13	0.0001
Error	28	0.91	0.03		
Total (corrected)	35	3.67			

Note. Overall mean (n = 36) = 1.864; $\sqrt{\text{MSE}}$ = 0.1802; CV = 9.67%.
[a]Computed using SAS® Proc GLM.