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A Review on Varciance Component Analysis: Interpretation of Deviation in Blend Uniformity Sampling

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Abstract: ***Blend uniformity analysis is performed to check the homogeneity or uniformity of a powder blend, which involves blending of active pharmaceutical ingredient and excipient. In Pharmaceutical industry, sampling process plays a major role. Pharmaceutical sampling is process of selecting a part of the pharmaceutical product (raw material, intermediate product, final product) from a whole for further analysis. It is also a regulatory requirement. Variations in a statistically based sampling plan can be identified using Variance Component Analysis, where deviations intra/within locations or inter/between location results are identified and if intra location variation is identified it is known as a sampling error, whereas, if inter location variation is identified, it indicates non-uniformity of the blend. Usually, the final composition of the dosage form is just as uniform as the powder mixture used to make it. When creating formulations and processes, it's crucial to establish reliable blending and transfer techniques since they stop post-blending segregation of the mixture and allow for the production of commodities with a tolerable level of compositional homogeneity. Variance component analysis is a valuable technique to identify the underlying causes of mix and content uniformity issues, particularly during formulation and process development. Thanks to the use of statistically based sample strategies and proper sampling procedures, the right data are collected to allow for efficient statistical analysis. The examination and mitigation of potential underlying causes may lead to process improvements.***

Keywords: Variance component analysis, blend uniformity, content uniformity, sampling

1.Introduction

Pharmaceutical Sampling refers to the procedures used to choose a certain amount of a pharmaceutical product. The sampling method should be suitable for the sampling's goals, the controls that will be used on the samples, and the material being sampled. [1] A sample in the pharmaceutical setup is known as a portion of a substance that was obtained using a predetermined sampling method. Any sample should be big enough to support all planned testing methods, including repetitions and retention samples. The inspector should note that the sampled material is the only sample that is currently available (see Sampling record) if the amount of material is insufficient for the intended analyses and the retention samples. The evaluation of the results should also take into account any limitations brought on by the small sample size. By using the sampling procedure, one can create a sample from a single lot or batch. [1] Sampling may be done for a number of reasons, including, but not limited to, acceptance of a batch or lot, acceptance of a consignment, pre-qualification, in-process control, batch release testing, retention or control sample with drawl, identity verification, and adulteration. A system of quality assurance includes sampling as a key component. [2]

In Pharmaceutical industry, sampling process plays a major role. Sampling is having regulatory requirements and also from business point of view, a Pharma industry has to test a lot or a batch in the testing laboratories before getting release the lot or batch in to the market. Several problems arise in the Pharma industries many a times due to wrong techniques used during sampling. So, Pharma industries apply much stress in preparing a Standard operating procedure (SOP) or protocol and training of concerned persons on sampling. [2]

Sampling could be necessary for a number of different reasons, including prequalification, consignment acceptance, batch release testing, in-process control, special controls, inspection for customs clearance, degradation, or adulteration, or getting a retention sample.

The tests that will be run on the sample could be:

- An identification check.
- Comprehensive pharmacopoeial or equivalent testing.
- Special or customized tests.
- In order to:
- Avoid contamination of the opened container, the materials, and the operator;
- Prevent cross-contamination by other materials, products, and the environment;
- Safeguard the person taking the sample (the sampler) during the sampling procedure.

While this won't always be practicable when samples need to be obtained from a production line (such as in-process control samples), sampling should be done whenever possible in a space or booth that is specifically intended for and used for this purpose. In the sampling record, the location where the sample was taken should be noted. A sequential log of all the materials sampled in each region should also be kept.

It might be challenging to sample from huge containers of raw materials or bulk goods. To lessen the danger of cross-contamination and to prevent contamination of the sample

or the products still in the container (e. g., by dust), this task should be done wherever feasible in a separate, closed cubicle within the warehouse.

For example, when sampling items like aerosol valves, hormones, and penicillins, contamination with dirt or environmental particles should be avoided, special or dedicated conditions should be used.

The original sales pack can usually be obtained as a sample from places like pharmacies or hospitals without any issues. However, the inspector must make sure that the amount of sample taken is adequate for the intended analyses and for the retention samples, and that all of the units sampled are from the same batch and, ideally, the same location.

A formal procedure outlining the sampling process should exist. Details about the sampling's health and safety implications should be included. It should make sure that sufficient representative samples are collected for testing in compliance with requirements. Preferably, labels and closures should be able to detect unlawful opening. Never put back samples into the bulk.

The sample procedure should be properly overseen and recorded. The sample process should be carried out in a way that makes it possible to identify material non-uniformity. Pay close attention to any indications that the material is not conforming during sampling. Different shapes, sizes, or colors of particles in crystalline, granular, or powdered solid substances are indications of non-uniformity, as are wet crusts on hygroscopic materials, deposits of solid pharmaceutical product in liquid or semi-liquid goods, and stratification of liquid products. Such modifications, some of which would be easily reversible, can take place during extended storage or exposure to extremely hot conditions while in transit. The above-mentioned homogeneous portions of the material or bulk should be sampled and tested separately from the other component of the material, which has a normal appearance. [3]

It is best to avoid combining samples from various parts because doing so could disguise quality issues like contamination or low potency.

The batch number and, if known, the container number from which the sample was obtained, the amount collected, and the purpose for which it was taken should all be included on sample labels. At the moment of sampling, labels ought to be applied. The sample storage container should be appropriately labeled with the necessary information, including the sample type, name of the item, identification code, batch/lot number, code, quantity, sampling date, storage requirements, handling precautions, and container number. [4]

2.Types of Sampling in Pharmaceutical Industry

2.1 Random Sampling

Any container in a consignment or any piece of a container can be randomly selected when using this sampling technique.

For instance, any 10 containers can be chosen at random to serve as the sample group if only 10 out of 100 containers are to be examined. Random sampling from the top, middle, and bottom of any one of the ten containers is possible.

2.2 Systematic Sampling

With this sampling technique, samples can be taken at specific, regular intervals. Time or a number may be the interval.

For instance, if 10 containers out of 100 are to be sampled, each container can be chosen after every 10th container, such as after the 10th, 20th, 30th, and so on containers. In order to gather a composite sample of a specific batch as it is being processed, sampling can be done at regular intervals like 30 minutes, 60 minutes, or 120 minutes. This is another case where a specific time interval can be chosen as a way of systematic sampling. [10]

2.3 Stratified Sampling

Stratified sampling is the technique of taking representative samples from dosage units at predetermined intervals from points in the compression/filling procedure that are most likely to produce test results with extreme highs and lows.

"Stratified" refers to dividing into smaller groups (Strata). The strata with the highest likelihood of failure should be identified as the worst possible sampling sites. We can state that the entire batch has met the acceptance requirements if the samples taken from the strata meet the predetermined standards. Typically, stratified sampling is carried out during product validation or Process performance qualification (PPQ) of processes.

In that systematic sampling is done for regular batches while stratified sampling is done in PPQ or validation batches, the regular interval used in systematic sampling differs slightly from that used in stratified sampling. In systematic sampling, a composite sample is prepared while in stratified sampling, individual samples taken at regular intervals are individually analyzed.

An illustration of stratified sampling During the compression or capsule filling operation, collecting 7 dosage units from at least 20 sites or 3 dosage units from at least 40 locations. [12]

2.4 Pooled Sampling

Pooled sampling refers to grouping or taking a sample from a sample that has already been taken.

For instance: Let's say that, following batch compression, 100 dose units are needed in order to undertake an analysis of a product. And let's say that a sample of 1000 dosage units is taken as part of a systematic sampling, which involves taking samples either at regular intervals or from a

certain container number. One hundred units are randomly selected from a sample of one thousand dose units for laboratory analysis. So, as an example of pooled sampling, a composite sample of 100 dosage units is taken from a composite sample of 1000 dosage units. [2]

3.Types of Sampling Tools

3.1 Scoops

Using a spatula or scoop, small containers of solid materials can be accurately sampled. The samples are then combined to create a sample that is representative of that container. The suggested scoop designs, which should ideally be rounded, are shown in Figure 1.

Large particles will roll off if the scoop is too tiny for the sizes of the particles being sampled, and testing bias may be introduced. If the scoop is too large, on the other hand, an excessively large sample will be acquired for a specific number of increments.

It is best to transfer a scoopful of sample to the sample container in a single motion. It is best to avoid touching the scoop when removing medicinal products because this could separate the sample.

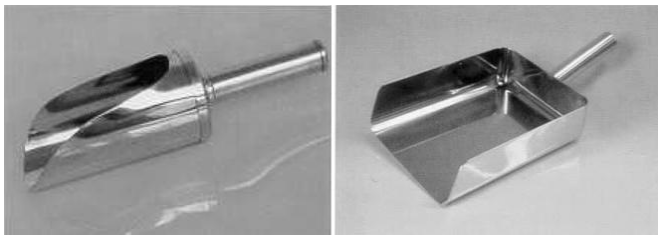


Figure 1: Designs of Scoops used for Sampling

3.2 Dip Tubes

Dip tubes, which should be constructed of an inert material like polypropylene or stainless steel, should be used for sampling liquid and topical medicines. Figure 2 depicts a common dip tube.

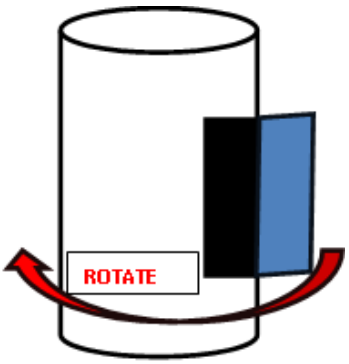
3.3 Sampling Rod

Sample a part of a material that was gathered using a certain sampling technique. Any sample should be large enough to accommodate all planned testing processes, including repetitions and retention samples. The inspector should note that the sampled material is the available sample (see Sampling record) if the amount of material is insufficient for the intended analyses and the retention samples. The evaluation of the results should also take into account any limitations brought on by the small sample size.

4.Overarching Factors

Sampling is the process of choosing a portion of a medicinal product for a specific use; for a definition, see the glossary. The sampling method should be suitable for the sampling's goals, the controls that will be used on the samples, and the material being sampled. The process needs to be documented in writing. Sampling-related tasks should be carried out carefully and with the right instruments and equipment. The validity of the subsequent studies is likely to be compromised by any contamination of the sample by dust or other extraneous material.

- In order to make it possible to collect samples from cohesive and moist powders, the Cohesive Pocket Sampler was specially modified from the Pocket Sampler for free-flowing powders.
- To help the powder attach to the sample pockets, scrapers have been added. Scrapers are completely welded to produce a GMP-compliant, hygienic sampler.
- Multilevel discrete sample type Construction made of 316 stainless steel.
- Sampling technique: The portion of the sampling procedure that focuses on the technique recommended for taking samples.



The typical sampling rod is made up of two concentric tubes; the inner tube is solid aside from the chambers where the sample is collected. The inner tube's chambers can be aligned with apertures in the outer tube, which is hollow. The powder bed will be least disturbed by a well-designed rod's sharp end.

A Sampling rod will deform the bed by bringing pharmaceutical substance from the blend's higher layers to its lower layers when it is put into a static powder blend. Whether the rod is introduced into the blend smoothly, jerkily, or twisting can affect how much of a distortion results. Therefore, it is important to design the proper sample procedure and train workers on how to use it. Sampling error can also be influenced by the angle at which the burglar enters the powder bed. In contrast to samples

that would be obtained with the same thief inserted at an acute angle, samples of varied particle sizes can be extracted when the thief is placed vertically into the powder bed. [5]

Generally sampling is done by introducing the sampling rod at an angle of 45º at the location and moving the sampling rod sideways for proper filling.

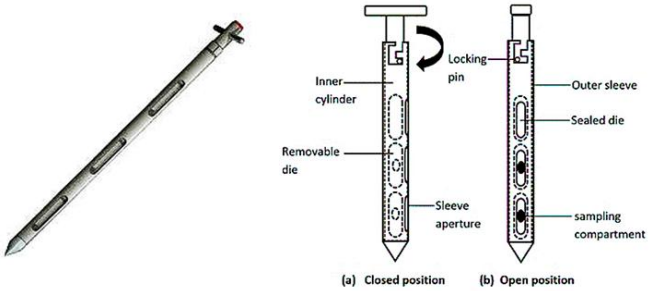


Figure 2: Sampling Rod. Showing Sampling Rod in Open and Closed Position

Dies: Dies are inserted in the sampling rod and are used to collect the required amount of sample. The size of the die may differ according to the amount of sample required.

The available die sizes are:

- 0.2 ml (minimum size 0.2 ml)
- 1 ml
- 1.5 ml
- 2 ml
- 2.5 ml
- 3 ml
- 3.5 ml
- 4 ml
- 4.5 ml (maximum size 4.5 ml)

Table 1: Sampling Plans for Exhibit and/or Process Validation Batches

Blend	Dosage Unit
Choose at least 10 areas in the blender from which to sample the blend. Locations that could show areas of poor mixing must be carefully selected. As an illustration, samples in tumbling blenders (such as V-blenders, double cones, or drum mixers) should be chosen from at least two depths along the blender's axis. A special effort should be made to perform uniform volumetric sampling for convective blenders (such a ribbon blender), encompassing the corners and discharge area (at least 20 locations are recommended to sufficiently validate convective blenders). From each location, collect at least three replicate samples.	Choose at least 20 sites to collect dose units throughout the compression or filling process. The sampling locations must be carefully selected to include samples from the start and finish of the compression or filling operation as well as critical events (such hopper changeovers) during the process. At least 7 dose units must be taken from each location.

5.Blend Uniformity Analysis

The final composition of the dosage form is typically as homogeneous as the powder mixture that was blended to create it. Building trustworthy blending and transfer techniques is essential when developing formulations and processes because they prevent post-blending segregation of the mixture and enable the manufacture of goods with a tolerable degree of compositional homogeneity. Thorough sampling should be done on both the blender and intermediate bulk containers (IBCs), when applicable, to establish an appropriate range of blending times, dead spots in blenders, segregation in IBCs, and the presence of sample error.

Blend sampling plans should be made so that variance component analysis can be used to quantify the variability that can be ascribed to the blend's homogeneity as well as any potential sampling mistake. If the **between-location error** in the blender is high, the blending operation's flaws must be fixed. [6]

The dose units should also be selectively sampled and tested in addition to the blend, with samples being taken at certain times and locations all along the compression or filling process. Throughout product development, comparisons between mix and dosage unit data should be made. Any inconsistencies between the blend and dosage unit uniformity data that are found should be investigated to determine the likely causes. [7]

An evaluation of the homogeneity of the powder blend and in-process dose units should be performed throughout the production of exhibit and/or process validation batches. However, utilizing blend data alone to confirm blending operations is exceedingly challenging for some goods due to sample mistakes. It is suggested that, in cases where sampling error has been demonstrated to exist, in-process dosage unit data be used in conjunction with blend sample data to show blend homogeneity. When producing show and/or process validation batches, both blend sampling and dose unit sampling are suggested in accordance with the sampling protocols listed in Table 1. Prior to the production of the show and/or validation batches, sampling sites and acceptance standards must be determined. [8]

6.Variance Component Analysis

Variance component analysis can be carried out if a strict statistically based sample plan is put into place (either duplicated sampling for a blend or stratified sampling for a medicine product). Within-and between-location variations

can be used to separate variations in mix potency or content homogeneity of dose units. This article refers to the total variation as total variance or total standard deviation (i.e., the square root of the total variance), the variation between locations as variation between locations or variation within locations, and the variation within locations as variation within locations or standard deviation.

A recommendation to develop blend and dose unit sampling strategies and assess the information using suitable statistical methods, such as variance component analysis (VCA), to gauge the level of results variability. Significant events (start-up, end-of-run, and bin change-over samples), though in relative proportion to the batch's overall size, should be included in sampling plans. Plans for sampling that are more exacting may be necessary for drugs with low doses and/or high potencies. [9]

Based on amendments to the withdrawn draft stratified sampling guidance document, Figure 3 shows a flow diagram for the evaluation of the sufficiency of powder mix and dosage unit homogeneity throughout the fabrication of process design and process qualification batches.

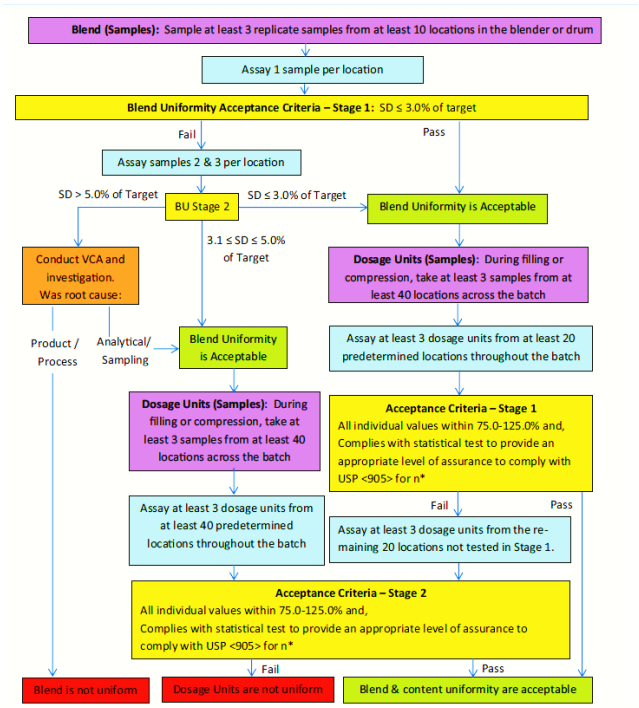


Figure 3: Process flow diagram for assessment of blend and content uniformity for process design and process qualification batches

Data on blend and medication product uniformity are useful to plot at the beginning of analysis since plots can reveal variability and trends in the data. There have been published scatter plots for scenarios that are frequently encountered. [13]

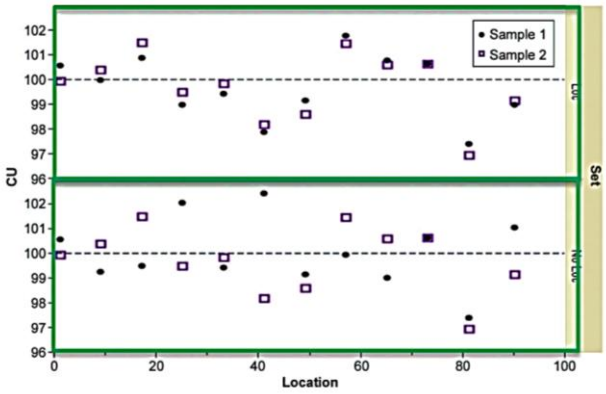


Figure 4: Batch Data with and without Variability

Standard deviation numbers are "rules of thumb" for the purposes of this article; standard deviations for potency (based on percent label claim) are regarded to be acceptable uniformity for both blend and drug product uniformity without additional examination when they are less than 3.0%. It may occasionally be overly analytical of the data to perform variance component analysis for batches with standard deviations less than 3.0%. Variance component analysis might be helpful to assist identify potential causes contributing to the non-uniformity if the total standard deviation is >3.0% for the blend and/or dosage units. It should also be mentioned that the inquiry should contain data from several lots of blends and dosage units. [14]

Blend homogeneity is adequate when the blend standard deviation is between 3.1 and 5.0% of goal, at which point in-process dosage unit sampling can be done. To analyze between-location and within-location variance components, which may be used to identify chances to enhance blend uniformity for subsequent batches, the group advises doing VCA on the blend data.

Conduct an investigation (including VCA) to see if the variability was caused by a non-blending problem that led to a false assessment of the true uniformity of the blend (such as sampling bias, analytical error, or other non-formulation/process causes) if the standard deviation is greater than 5% of the target. Corrective steps should be taken and the batch can move on to stage 2 dosage unit testing if the existence of such an issue can be proven and supported. If the high standard deviation is instead attributable to a source that is related to the product or the manufacturing process, the blend homogeneity is undesirable, and the product will need to undergo further formulation and/or process development.

Every time stage 2 dose unit testing is necessary, a VCA of the data is advised to see if there are any location-to-location difficulties during the compression or filling run. It is useful to compare the VCA results obtained for the mix to those obtained for dose units. These studies' findings might shed light on how to improve mix and content uniformity. [14]

7.Conclusion

The primary objective of blend uniformity analysis is to show that, as indicated by the standard deviation of blend data, the medicine is uniformly distributed throughout the mix. Although the mean across all samples is given, sampling bias frequently causes the value to be inaccurate. For instance, a lower, albeit consistent mean may be produced by the preferred flow of excipients (and the inferior flow of the drug ingredient) into the chamber of a sample thief. Because of this, the only assay value to be concerned about is the mean of the dose units tested (either during in-process testing or during release testing).

Together, blend and content consistency should be evaluated. A thorough examination of the batch's data and information as well as the product's past should be included in the assessment. A new lot of material, a modification to the drug substance's synthesis, or a change in the operators sampling the blend, for example, could have introduced variability into the process and should be investigated in batches that differ from historical data.

To evaluate the spread and any trends in the data, data should be plotted for the mix and dosage units from each location.6 The consistency of the results should be assessed by comparing the means, standard deviations, and variance components for the mix and dose units side by side. In general, the blend and dose forms can be regarded as uniform if the standard deviation is less than about 3%. To find possible areas for process improvement, standard deviations greater than 3% may be subjected to variance component analysis.

A large between-location variance component for the blend suggests that there has been insufficient macroscale mixing because the blend is not consistent throughout all locations of the blender. A significant between-location variance component for tablets suggests that the dose units are not constant throughout the entire compression or filling process.

High within-site variance components indicate variations in the assay values for replicate samples within a single location for both the mix and dose units. Additionally, high within-location variance components may be a sign of biased sampling.

In particular during formulation and process development, variance component analysis is a useful method to pinpoint the underlying reasons of mix and content uniformity problems. The right data are collected to allow for effective statistical analysis thanks to the use of statistically based sample strategies and appropriate sampling methodologies. Process improvements may result from the investigation and mitigation of potential underlying causes.

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