

# Development and Validation of a Novel Process for the Production of Powdered Cell Culture Media

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## Abstract

A high throughput air classifier mill (ACM) has been combined with vertical ribbon blending technology to provide a novel continuous flow manufacturing process for the production of powdered media. This process provides the flexibility to generate quantities from 40 kg to 4,000 kg of powdered media in a single operation. This presentation will focus on the analysis of powder blends to validate the uniform distribution of medium components while in-process and in finished product. In these studies, samples were withdrawn by means of a sample thief from several areas of the blenders as well as from storage containers of discharged medium. Process development studies were performed using a model dry blend of components ranging from 10 percent to 1 part-per-million. The distribution of glucose, amino acids, and inorganic species in several standard media formulations was also evaluated for validation batches ranging from 50 kg to 4,000 kg.

## Introduction

The validation of blending operations has long been recognized as a critical aspect in the manufacture of pharmaceutical solid dosage forms. Cell culture media are classified by the FDA as a class I medical device. The validation of manufacturing processes for this class of product is required by 21 Code of Federal Regulations (CFR) Part 820. While the requirements are defined by these regulations in broad terms, it states that the process validation program for a powdered cell culture medium be defined, and implies that it should include a component that focuses on the blending operation.

The mixing and flow properties of powder blends are influenced by particle size, particle shape, density, and particle size distribution. The most important of these effects is considered to be particle size. In order to achieve good content uniformity in a powder blend, it is imperative to control the particle size and particle size distribution of the blend components.

Traditionally, powdered cell culture media have been manufactured by ball milling operations. Ball milling is an inherently inefficient operation due to batch size limitations and the high degree of manual intervention required. The use of grinding media (or stones) introduced potential for cross-contamination of products and contamination of the product with pieces of the grinding material is a known complication of the method. Perhaps the biggest drawback to the ball milling operation as it relates to product homogeneity is the limited control over the particle size of the milled product components.

We have designed a novel system for the manufacture of powdered cell culture media based on two key pieces of process equipment. The first is the air classifier mill (ACM) which employs a grinding chamber, where components are pulverized by impactors fixed to a high-speed rotor, and a radial separator that allows fine particles through and redirects oversized particles back into the path of the impactor for additional size reduction. Particle sizing can be controlled with the ACM by increasing or decreasing the separator speed. The mill also sizes particles within a narrow particle size distribution by reducing over-grinding that can occur with ball milling.

The second key piece of process equipment is a vertical ribbon blender. Once powdered medium components leave the ACM, they are pneumatically fed via a closed system into either a 400 liter or 5000 liter vertical ribbon blender. The mixer is designed with a helical rotor that creates a three-dimensional flow that ensures high quality mixing with extremely gentle action. The vertical ribbon blenders offer many advantages over traditional blenders including the flexibility to operate at fill levels ranging from 10% to 100% of capacity; the ability to discharge up to 99.99% of the batch without segregation; and a pharmaceutical grade design and material contact surfaces.

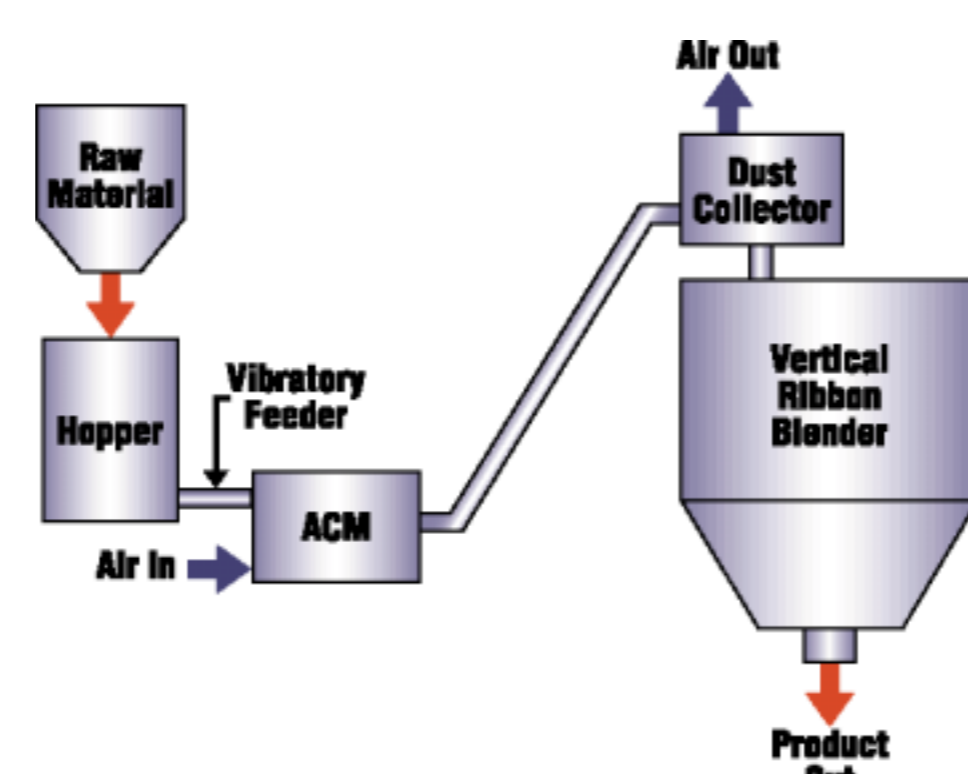
This communication will present the analysis of powder blends performed to optimize process parameters and to demonstrate the uniform distribution of powdered cell culture medium components while in-process and in the finished product.

## Materials and Methods

### Milling and Blending Operations:

An air classifier mill, model NSP-1, from Sturtevant (Boston, MA) was used to pulverize product components prior to blending. The mill was operated with main motor speed of 9000 rpm and a separator motor speed of 1200 rpm. A volumetric feeder was used to meter the flow of powder supplied to the mill. Powder discharged from the mill was transferred to a blender by a dust collector (Custom Systems) mounted over the blender. Blending of powders was performed in one of two vertical, single shaft ribbon blenders from Ruberg (Paderborn, Germany) depending on the scale of the batch. Blender model VM400 has a maximum fill of 400 liters and can efficiently blend powder loads ranging from 50 to 400 liters. Blender model VM5000 has a maximum fill of 5000 liters and can efficiently blend powder loads ranging from 450 to 5000 liters. The entire processing system is shown schematically in Figure 1.

Figure 1: Schematic diagram of the process system for milling and blending



### Sampling of Powder Blends:

Samples were withdrawn, with a thief, from ten different locations within the blender. The sampling thief consisted of two concentric tubes, an inner tube that was solid except for a single chamber designed to allow for the collection of a 5 gram sample, and a hollow outer tube that contained an opening that could be aligned with the chamber of the inner tube after insertion into the powder bed.

Five sets of samples (i.e. five thief stabs) were collected from each of the ten locations. For the model blend studies, the blending run was interrupted to allow for intermittent sampling at five, ten, fifteen, twenty, and thirty minutes. An additional sampling was conducted after forty minutes for the VM5000 blender studies. For process validation studies, blender samples were obtained at 20 and 30 minutes in the VM400 blender and 30 and 40 minutes in the VM5000 blender. In addition, ten sets of samples were collected from storage containers (i.e., 50 kg drums) post-discharge from the blender. One sample from each set at each location within the blender at each mixing time and one sample from each location with the storage containers were submitted to our QC Laboratories for analysis. The remaining samples were saved as retains for further analysis as necessary.

## Results and Discussion

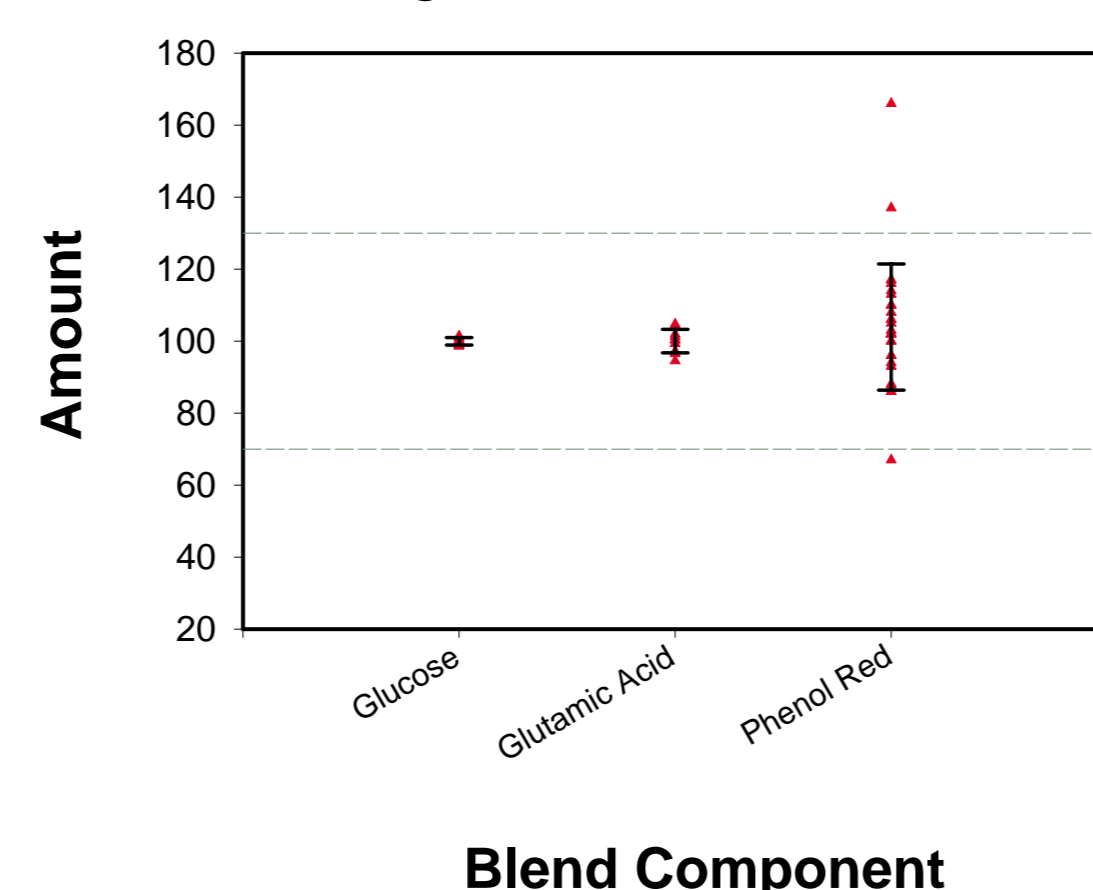
Powdered cell culture media are a very complex blend of components including amino acids, vitamins, carbohydrates, inorganic salts, and biological buffers. The cost of raw materials are significant and trace component analysis is difficult due to the complex sample matrices. An initial process optimization program was developed based on a model blend with a composition reflective of typical powdered cell culture medium.

A direct mix dry blend composed of 10% glucose, 0.01% glutamic acid, 0.0001% phenol red, and 90% sodium chloride was used as the model system. The materials chosen for the model blend are significantly less expensive than those present in a finished product and the simplified sample matrix allowed for robust analytical procedures to be developed for trace component analysis.

The content uniformity results for a 350 kg batch of the model blend after an initial five minutes of mixing is shown in Figure 2. The assay value of the sample from each location, expressed as percent label claim, is represented by a single triangle. The standard deviation of the sample set is also displayed in the figure. The data clearly demonstrate that excellent content uniformity of the glucose component is rapidly established in the blender. Even the glutamic acid component, present at 0.01%, is distributed sufficiently to satisfy the content uniformity specifications (85.0 – 115.0% with a relative standard deviation value of < 6%) set for glucose and glutamic acid.

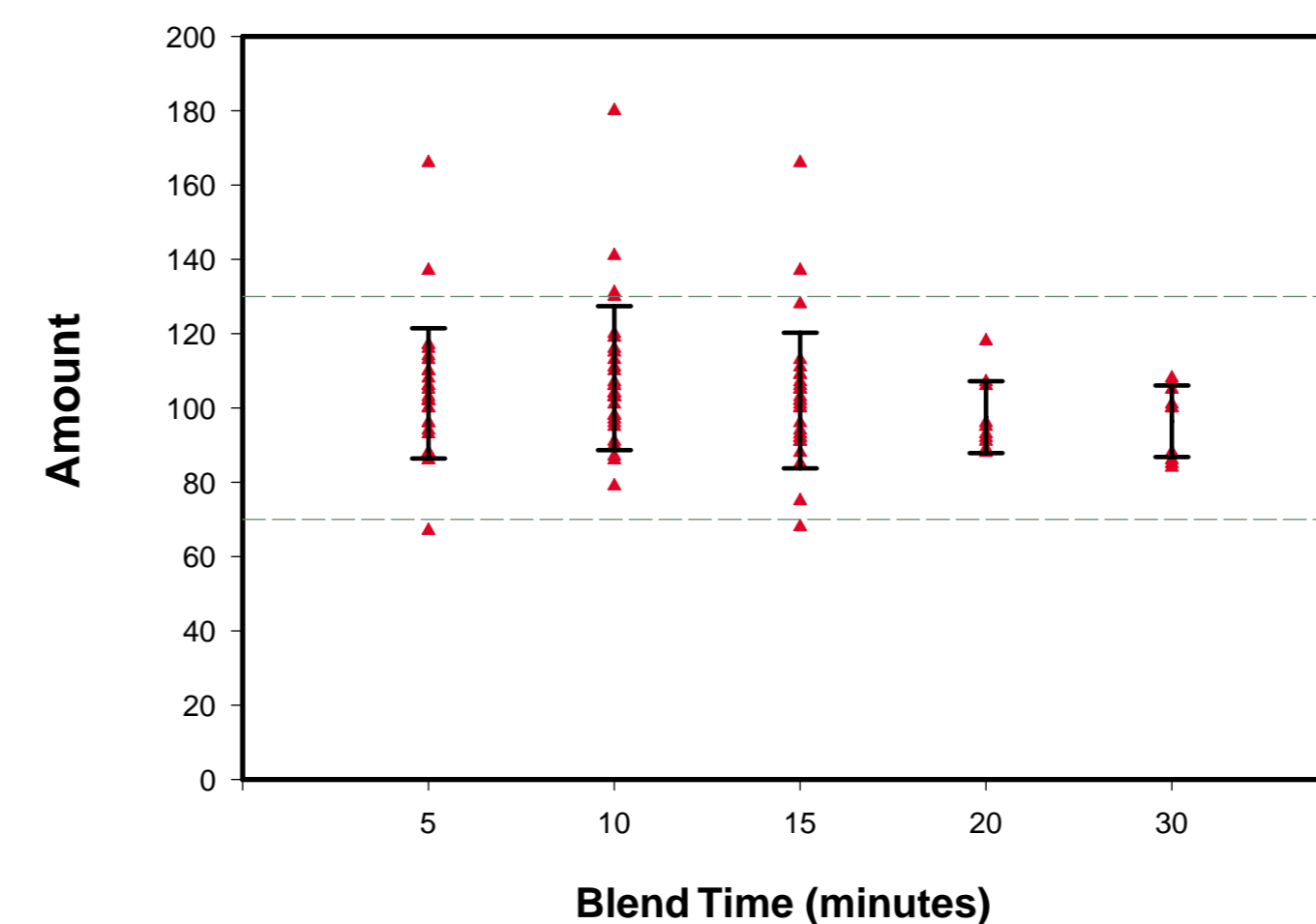
The content uniformity specification for phenol red, present at 0.0001%, was established *a priori* at 70.0 – 130.0% with a relative standard deviation (RSD) value of < 12%. Analysis of the first set of ten samples from the five minute mixing time indicated a single assay value outside the acceptance range (67% of label claim) and an RSD of 13%. An additional twenty samples were tested from the five minute mixing time, following the two-tier testing guidelines established by USP Uniformity of Dosage Units Criteria [1]. Two additional outliers were identified from the second set of samples and the RSD increased to over 16%, as seen in Figure 2.

Figure 2: Content uniformity of 350 kg model blend after five minutes of mixing in VM400 blender.



The mixing of the 350 kg batch of the model blend was continued and samples collected intermittently after ten, fifteen, twenty, and thirty minutes of mixing. Glucose and glutamic acid continued to show excellent content uniformity at all mixing times. The effect of mixing time on the trace phenol red component is shown in Figure 3.

Figure 3: Effect of mixing time on the content uniformity of 0.0001% phenol red in a 350 kg model blend in model VM400 blender.



The data in Figure 3 demonstrate a dramatic improvement in content uniformity after 20 minutes of mixing which is maintained for up to 30 minutes of mixing. All ten samples assayed at 20 minutes and 30 minutes were within the acceptance range with RSD values of 9.9% and 9.8%, respectively.

Additional blending development studies were also conducted using the model blend with batch sizes of 50 kg in the VM400 blender, and 450 kg and 4500 kg in the VM5000 blender. The content uniformity specifications for all components were satisfied for the final two mixing times sampled for all batches (20 and 30 minutes in VM400 blender; 30 and 40 minutes in VM5000 blender).

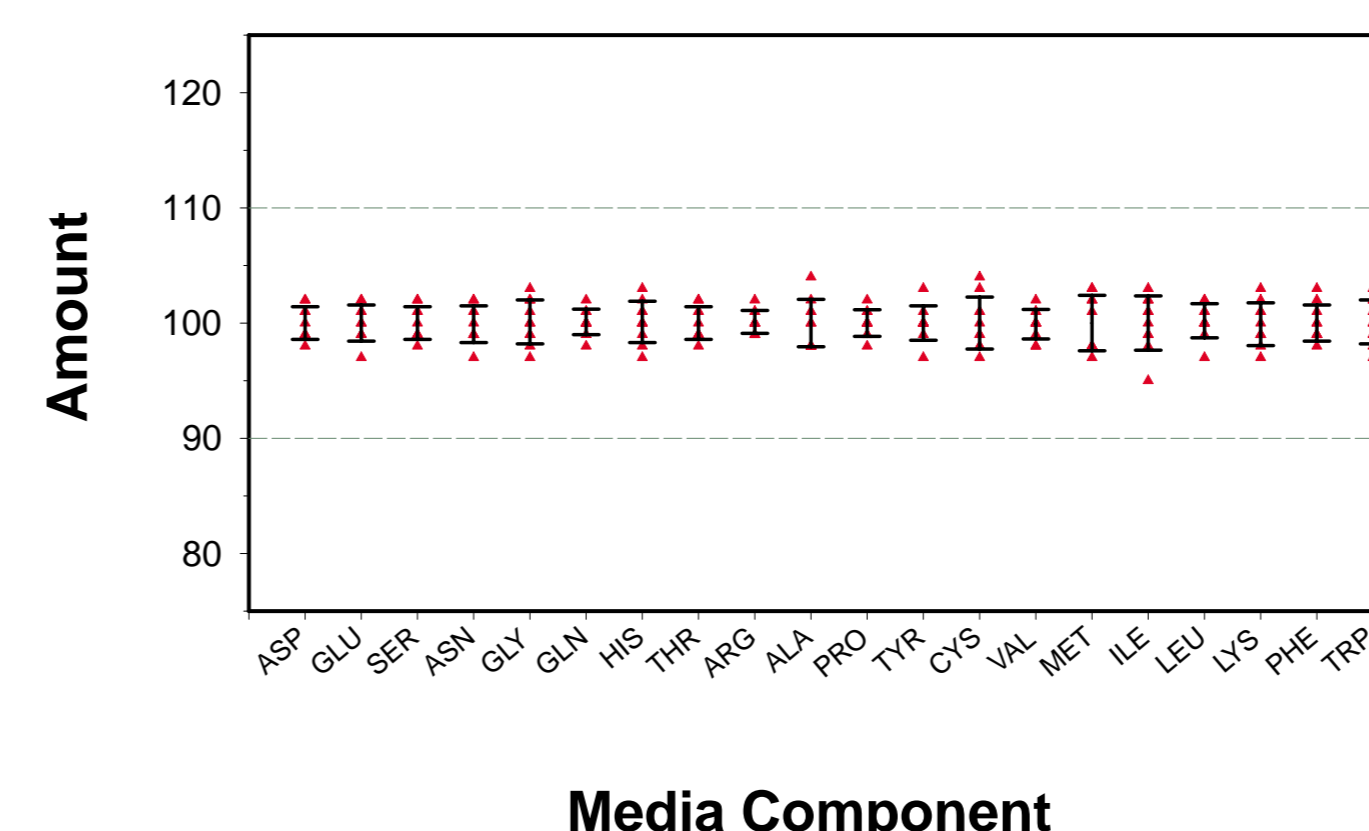
Based on the data obtained from the studies of the model blend system, process parameters for the blending of powdered cell culture media were established. The working capacities of the VM400 and VM5000 blenders were defined as 50 to 350 kg and 450 to 4500 kg, respectively. Blending time ranges for the VM400 and VM5000 blenders were defined as 20 to 30 minutes and 30 to 40 minutes, respectively.

A program was developed to validate the manufacturing process for powder cell culture media. Validation runs were scheduled at the lower and upper working capacity of each blender, as well as at an intermediate batch size for each blender. Due to the high number of individual medium formulations to be manufactured with this system, the validation plan was based on a matrix approach, mandating the milling and blending of three media representative of typical products that would be manufactured with this system. The three media selected to represent a cross-section of anticipated products were MCDB-105 (Sigma product M6395), RPMI-1640 (Sigma product R6504), and DME/F12 (Sigma product D8900). The formulations of these products are available elsewhere [2-4].

All process validation samples were assayed for glucose content, amino acid content by amino acid analysis, and elemental concentrations by ICP-AES. Cell culture testing on two different cell lines was also performed on each sample. A single bulk sample from each run was characterized by bulk density, tap density, solution pH, endotoxin, moisture content, and particle size distribution.

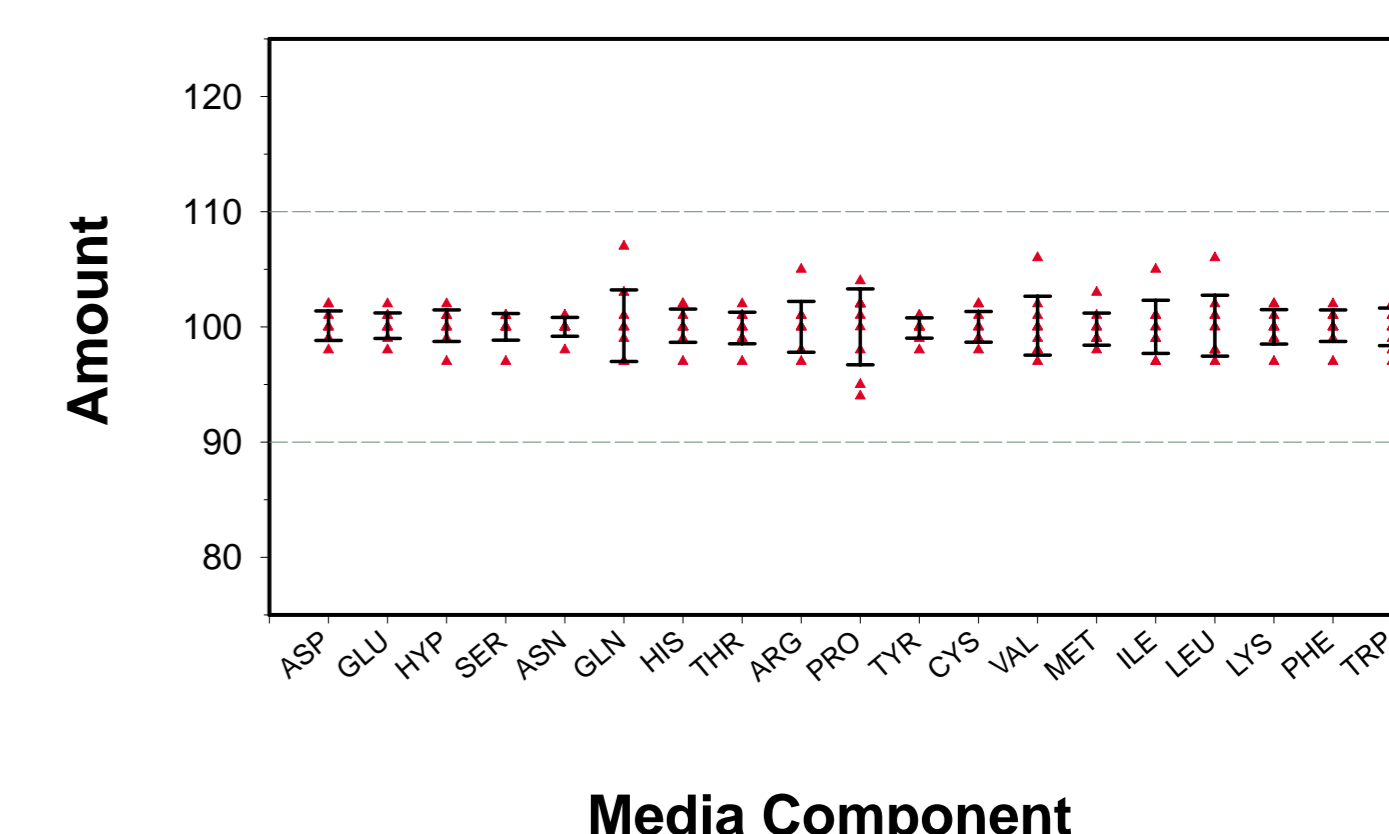
The content uniformity results of twenty individual amino acids for a 48 kg batch of MCDB-105 after twenty minutes of mixing is shown in Figure 4, expressed as percent label claim. The content of amino acids in this formulation range from 2.5% for L-glutamine down to 0.01% for L-tryptophan. The data in Figure 4 demonstrate excellent content uniformity for all twenty amino acids in this medium formulation. Content uniformity specifications were satisfied for blender samples taken from further qualification runs comprised of three medium formulations ranging from 48 kg to 4,000 kg.

Figure 4: Content uniformity of twenty amino acids in a 48 kg batch of MCDB-105 after twenty minutes of mixing in the VM400 blender.



Uniformity of a final blend does not guarantee uniformity of the finished product. Subsequent handling of the powder blend such as discharge from the blender into drums and hoppers provide ample opportunity for particle segregation. A credible process validation must demonstrate acceptable content uniformity of the final blend and finished product. The content uniformity results of twenty individual amino acids for samples taken from product storage containers of a 3,980 kg batch of RPMI-1640 is shown in Figure 5, expressed as percent label claim. The content of amino acids in this formulation range from 2.9% for L-glutamine down to 0.05% for L-tryptophan. The data in Figure 5 demonstrate excellent content uniformity for all twenty amino acids in this medium formulation. Content uniformity specifications were satisfied for drum samples taken from further qualification runs comprised of three medium formulations ranging from 48 kg to 4,000 kg.

Figure 5: Content uniformity of twenty amino acids in a 3,980 kg batch of RPMI-1640 after discharge into product storage containers.



Media Component

## Conclusion

A novel system for the milling and blending of powdered cell culture media has been established with the combination of an air classifying mill and vertical ribbon blenders. Process parameters were established and demonstrated to achieve product homogeneity down to the 0.0001% level in a model powder blend. Process validation studies conducted at a production scale ranging from 50 kg to 4,000 kg verified content uniformity of over twenty individual medium components in the final blend as well as after discharge into product storage containers.

## References

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