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Application Note

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Characterization of Filling Accuracy Using the Linkit® AX Aliquoting Solution

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Abstract

In this characterization study, aliquoting operations were conducted using the Linkit® AX Aliquoting Solution. The primary objective was to evaluate and identify the factors that exert the most significant influence on the accuracy of distribution and filling using a design of experiments (DoE) approach.

The following factors were studied:
- Filling process parameters
  - Filling speed
  - Air purging speed
  - Slow down at (% total target weight at which filling speed is reduced)
  - To final speed (the speed maintained until total target weight is reached)
- Total weight filled in bag containers: 1,500 to 10,000 g (equal to 150 to 1,000 mL of water in each of the ten bags)
- Fluid viscosity (0.9 to 5.4 cP) and temperature effects
- Number of bags filled

Filling speed and the total weight filled in bag containers were identified as significant factors. In comparison, the impact of the fluid to be aliquoted and the quantity of bags to be filled was negligible. The higher the filling speed, the more accurate the aliquoting. For total weight in bags between 5,000 and 10,000 g, the optimized filling parameters were 250 rpm filling speed with a slow down at 99% full (i.e., the speed is reduced when the bag reaches 99% total target weight to a speed of 150 rpm). For total weight in bags ≥ 1,500 and <5,000 g, the optimized filling parameters were 250 rpm filling speed, with a slow down at 80% full to a speed of 150 rpm.

Distribution accuracy was also calculated under different conditions; 4Cell® Nutri-T media was aliquoted, with different total weight values filled in bag containers and with or without a filter between the pump and Linkit® AX single-use assembly. The results show no significant difference between 4Cell® Nutri-T media and water. The worst results obtained were with a total weight filled in bags of 1,500 g, and there was no significant impact of the presence of a filter as long as the same flow rate at the main inlet line was maintained.

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Introduction

When performing an aliquoting operation on a manifold of containers, the accuracy between the different collection containers is a key performance characteristic.

Filling performance for the Linkit® AX can be determined at the level of:

1. Single product – distribution accuracy between each of the filled bags.

To determine the distribution accuracy for single units, each bag was weighed before and after filling using the following equation:

$$\text{Distribution accuracy between all filled bags of a single unit [\%]} = \frac{\frac{\text{Max - Min}}{2}}{\text{Average weight}}$$

(max = the heaviest bag and min = the lightest bag of a Linkit® AX single-use assembly)

2. Single lot – filling accuracy between bags of all Linkit® AX products within a lot.

The filling accuracy for a lot considers all bags in a given configuration and is calculated based on the maximum difference observed between the bag weight measured after filling:

$$\text{Filling accuracy between all bags in a lot [\%]} = \frac{\frac{\text{Max - Min}}{2}}{\text{Average weight}}$$

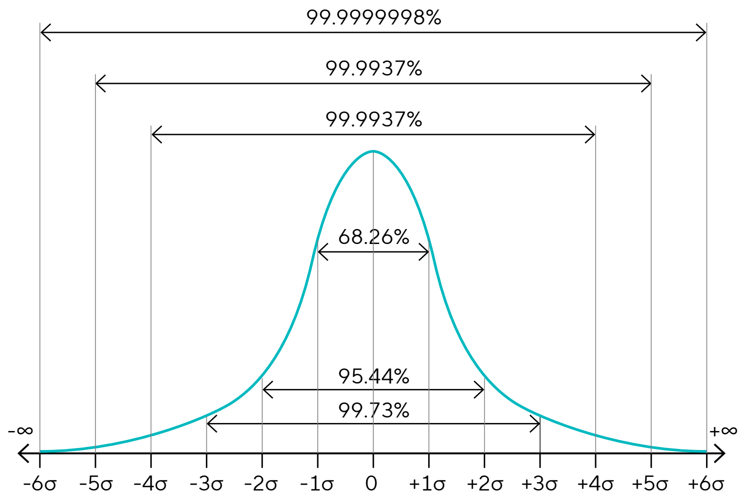
(max = the heaviest and min = the lightest of all filled bags)

3. Multiple lots – filling accuracy determined by predictive analysis modeling

This statistical predictability analysis considers worst-case data and added batch-to-batch variation in production, the different configuration possibilities, and the tested process parameters. The statistical approach chosen uses different sigmas (Figure 1 and Table 1).

It is important to note that distribution and filling accuracy performances are a multi-factor variable: the equipment and consumables, process parameters, fluid properties, environmental conditions, and operator can all impact their performances.

Figure 1: *Representation of the Statistical Approach*



Note. **1.** The filling accuracy value at 1σ means that, in the worst case, 68.26% of Linkit® AX products will meet this filling accuracy value, and the other 31.74% of products might have a greater value. **2.** The filling accuracy value at 2σ means that, in the worst case, 95.44% of Linkit® AX products will meet this filling accuracy value, and the other 4.56% of products might have a greater value. **3.** The filling accuracy value at 3σ means that, in the worst case, 99.73% of Linkit® AX products will meet this filling accuracy value, and the other 0.27% of products might have a greater value.

Table 1: *Results Obtained During Validation Conditions*

Distribution Accuracy	Single unit	150 mL	± 6.5%
		250, 500, 1,000 mL	± 5.1%
Filling Accuracy	Single lot	150 mL	± 7.7%
		250, 500, 1,000 mL	± 5.9%
	Multiple lots	150 mL	± 8.7% at 3σ
		250, 500, 1,000 mL	± 7.3% at 3σ

Note. Water was aliquoted at room temperature, with optimized parameters.

The purpose of this application note is to determine the factors significantly impacting distribution and filling accuracy when using the Linkit® AX solution and provide guidance on how to reach optimal distribution and filling accuracy.

Note that the Linkit® AX solution has been specifically designed to minimize the influence of equipment and operator interactions on accuracy. It is composed of the different items detailed in Figure 2.

All findings presented in this application note were obtained using the complete Linkit® AX solution. If the single-use assembly is used with a different equipment set, the influence of the operator or the equipment itself could significantly negatively affect accuracy.

Figure 2: *Linkit® AX Aliquoting Solution*

Part 1 – Identifying the Key Factors Affecting Performance Accuracy

Materials

- Linkit® AX Aliquoting Solution (Figure 2) composed of:
  - Sartorius customized Watson Marlow 630 DUN/R peristaltic pump
  - Cubis® II balance with Linkit® AX QApp
  - Purge Stand
  - Linkit® AX single-use assemblies of different volumes, 25-45 kGy gamma-irradiated

- Different fluids:
  - Sugar solutions of 15 and 30 wt%
  - Water
- Fluid temperatures: 4 and 45 °C

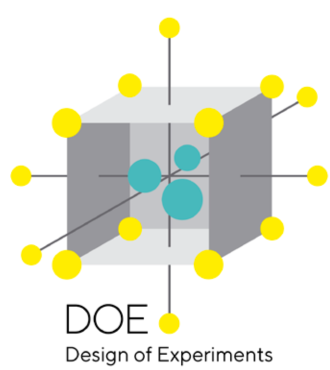
The sugar concentration and temperature resulted in a solution viscosity range from 0.9 to 5.4 cP during the tests.

Methods

A design of experiments (DoE) was used to define the test plan (Figure 3). The primary goal of a DoE is to streamline the test plan by using a mathematical model that encapsulates the entire design space, as defined by the factor limits. This method facilitates the assessment of each factor's influence on the variable of interest while optimizing the number of trials. Additionally, it assists users in evaluating the combined effects of multiple factors.

The factors (represented as an axis on the cubic representation in Figure 3) potentially impacting distribution and filling accuracy were defined. The model limits for each factor were then determined (minimum and maximum of the axis). For example, the pump speed cannot exceed a maximum pump speed value, constituting a maximum limit for this factor. The limit can also be set in cases where it is not possible to perform the testing or the result cannot be measured above or below a given value of a factor. For example, aliquoting cannot be performed with a fluid exceeding a temperature of 50 °C as it exceeds the specified operating temperature.

Figure 3: *DoE Representation*



The following factors and their limits were specified:

Factor	Limit
Air purging speed [rpm]	0–50
Filling speed [rpm]	10–250
Slow down at [%]	80–99
To final speed [rpm]	10–150
Fluid concentration [% wt] (sugar solution to represent fluid viscosity)	0–30
Fluid temperature to play on fluid viscosity [°C]	4–45
Total filled weight in bag containers [g]	1,500–10,000
Number of bags filled	5–10

Note, for total weight in bag containers under 1,500 g (<150 mL of water in ten bags), design limitations severely impact distribution and filling accuracy as the pump does not have enough time to start and stop before reaching the target weight.

The model linearity was also checked using center points for each factor. The output variable was the weight standard deviation. To determine this output variable, each of the filled bags per product was weighed after the aliquoting operations. The weight standard deviation is directly linked to distribution and filling accuracy.

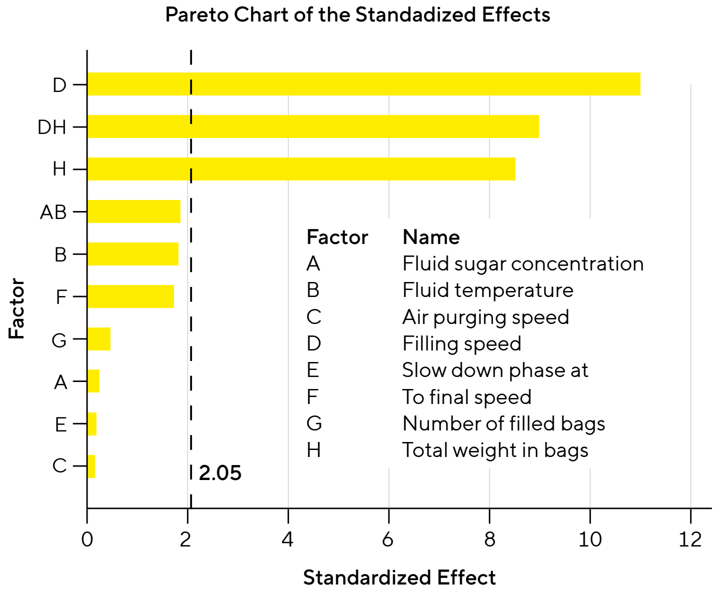
A DoE test plan was determined and performed. The results were entered into the model, enabling the most significant factors to be identified and optimization strategies to obtain the best filling performances.

Results

The model output can be observed in Figure 4, which illustrates the impact of each factor on the weight standard deviation (performance accuracy).

The standardized effect (horizontal axis in Figure 4) is a variable showing the influence of a factor on the model. The black value is the limit below which the factors are not considered significant. As can be observed, the most significant factor is the filling speed, followed by the combination of filling speed and total weight filled in bags, and finally, the total weight in bags itself.

Figure 4: *Pareto Chart Obtained With the DoE Showing Standardized Effects of Factors on Filling Accuracy*



The following factors all have an impact on performance but are insignificant compared to the filling speed and total weight in bags:

- Concentration
- Fluid temperature
- To final speed
- Number of bags filled (the assembly is available with ten bags but it is possible to close container inlet lines to fill fewer than ten bags)
- Air purge speed

The observation that fluid concentration (sugar concentration in this case) and temperature do not significantly influence accuracy means that fluid viscosity is also not a significant factor (since it is a product of concentration and temperature). In summary, we can conclude that the solution to be aliquoted does not significantly impact the filling accuracy within the tested range.

The filling speed bar in Figure 4 shows that the number of bags to be filled has a negligible impact on accuracy compared to the most influential factors. Nevertheless, if too many bag inlet lines are closed, excessive pressure can build up, creating a leakage risk at connection points. The Linkit® AX should not exceed a pressure build-up of 0.3 bar.

Impact of Significant Factors: Filling Speed and Total Weight Filled in Bags

Different total weight values filled in bags were tested; the results are summarized in Figure 5.

Figure 5: *Cube Plot (Fitted Means) for Liquid Volume Standard Deviation*

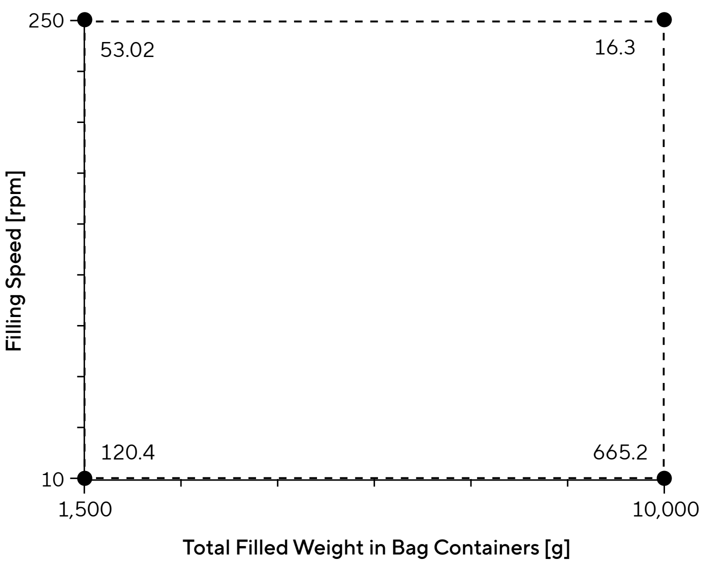


Figure 5 represents the impact of the total weight of liquid being filled in the bag containers (x-axis) and the filling speed (y-axis) on the weight standard deviation observed (values which are in the rectangle).

The first conclusion is that the higher the filling speed, the better the distribution and filling accuracy between the bags. For example, for a total weight of 1,500 g, i.e., 150 mL of water in each of the ten bags, the weight standard deviation varies from 120.4 g (with a filling speed of 10 rpm) to 53.0 g (with a filling speed of 250 rpm).

Therefore, filling speed is a significant factor and to reach the best distribution and filling accuracy, the highest filling speed must be used. If process or pump limitations are an issue, 250 rpm can be considered an optimal value. The same conclusions can be made for the 1 L bag (Figure 5).

The second conclusion is that once the filling speed is optimized, the higher the total weight to be filled in bags, the better the distribution and filling accuracy. The total weight filled in bags is correlated with the total target weight entered into the balance for the aliquoting operation.

A regression was then performed to optimize the filling parameters for each total weight range to reach the best distribution and filling accuracy. A linearity was observed with a filling speed above 130 rpm, depending on the volume of the bag filled. This means that the impact of the speed above 130 rpm is significant and evolves linearly. Under 130 rpm, there is no linearity; the impact of a slight change on accuracy can be very significant.

The model determined the optimized parameters for Linkit® AX using all ten bags:

Total Weight to be Filled in Bags [g]	Air Purging Speed [rpm]	Filling Speed [rpm]	Slow Down at [%]	To Final Speed [rpm]
≥ 5,000 (corresponds to 500 mL of water in each of the ten bags)	50	250	99	150
1,500 – 5,000 (corresponds to water volumes between 150 and 250 mL in each of the ten bags)	50	250	80	150

Conclusion

The DoE revealed that the most significant factors in distribution and filling accuracy are:

- pump filling speed
- total target weight

The higher the filling speed, the better the accuracy. Once set to the highest filling speed, the higher the total target weight, the better the accuracy. The impact of the liquid characteristics to be aliquoted and the quantity of bags to be filled are negligible in comparison to the filling speed and the total target weight.

Part 2 — Distribution Accuracy Study: Liquid Type, Total Weight Filled in Bags, and Filter Presence

Materials

- Linkit® AX Aliquoting Solution composed of:
  - Sartorius customized Watson Marlow 630DUN/R peristaltic pump
  - Cubis® II balance with Linkit® AX QApp
  - Purge stand
  - Linkit® AX single-use assemblies of different volumes
- Different fluids:
  - Water with 30% wt sugar
  - Water
  - 4Cell® Nutri-T GMP Medium
- Presence or absence of a Sartopore® 2 MidiCaps 0.2 µm size 9 filter between the pump and the Linkit® AX single-use assembly

The Linkit® AX solution fills ten Flexsafe® bags simultaneously. Filling performance was determined at the level of a single product — distribution accuracy between each of the ten bags. Multiple products were tested for each assay.

The filling performance trials were done at room temperature with different solutions using the optimized filling parameters described above. Table 2 shows the tested Linkit® AX products.

To ensure the most accurate conditions for weight results, each bag was disconnected at the connection level before filling, weighed, and re-connected. Filling was then performed, and bags were disconnected and weighed again to determine distribution accuracy.

Methods

Aliquoting operations were performed with the following optimized filling parameters:

- For total weight in bags ≥ 5,000 g:
  - Air purging speed: 50 rpm
  - Filling speed: 250 rpm
  - Slow down at: 99% full
  - To final speed: 150 rpm
  - Total target weight of 5,250 g for 5,000 g total weight in bags and of 10,250 g for 10,000 g total weight in bags.
- For total weight in bags between 1,500 and < 5,000 g:
  - Air purge speed: 50 rpm
  - Filling speed: 250 rpm
  - Slow down at: 80 – 99% full
  - To final speed: 150 rpm
  - Total target weight of 1,750 – 1,580 g for 1,500 g total weight in bags and 2,580 g for 2,500 g total weight in bags.

Results

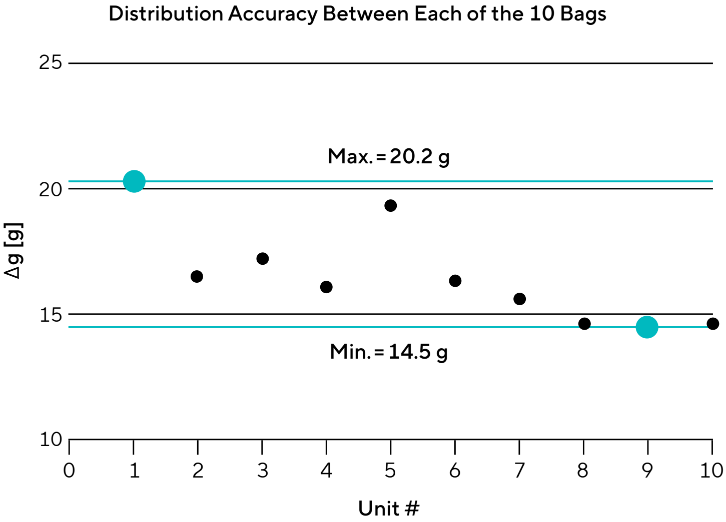
4Cell® Nutri-T Media
For trials with cell culture media, the aliquoting operation was performed with 4Cell® Nutri-T media at room temperature with the optimized parameters. The difference in grams (Δg) between the heaviest and lightest bag was recorded.
The details of the trials performed on configurations with 150 mL bags are given below. The same method has been applied for the rest of Part 2.

Figure 6 illustrates the distribution results of the ten trials with Linkit® AX configuration 150 mL filled with 4Cell® Nutri-T media. The total target weight was 1,750 g.

Table 2: *Tested Linkit® AX Products*

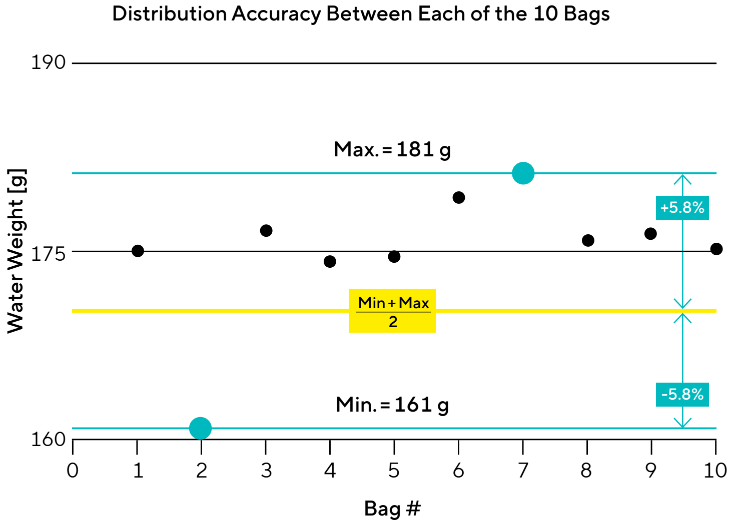
Single-Use Assembly	Linkit® AX						
Bag volume on Linkit® AX [mL]	150	500	500	500	500	1,000	1,000
Targeted total weight in bags [g]	1,500	1,500	2,500	5,000	5,000	10,000	10,000
Approximated equivalent volume in bags [mL]	150	150	250	500	500	1,000	1,000
Solution	4Cell® Nutri-T	Water		4Cell® Nutri-T	Water		Water with 30% sugar concentration
Filter	No						Yes
Temperature	Ambient (22±3 °C)						

Figure 6: *Distribution Accuracy of Linkit® AX 150 mL Configuration Filled With 4Cell® Nutri-T Media*



Note. Each data point represents a single product. Graph shows ten trials with ten bags.

Figure 7: *Distribution Accuracy Between Each of the Ten Bags in the Worst-Case Unit for 150 mL Configuration*



Note. Each data point represents a single bag.

A deep dive into the worst-case unit (Unit 1, Figure 6), where Δg = 20.2 g between the heaviest and lightest bag, shows a distribution accuracy of ± 5.8% vs. the average between min and max (Figure 7).

The summary of the results obtained with 4Cell® Nutri-T compared to the results obtained during validation with water can be found in Table 3.

No significant difference was observed between the accuracy of 4Cell® Nutri-T media and water aliquoting operations. In this set of trials, slightly better performances were obtained with 4Cell® Nutri-T media compared to the validation performed with water.

Table 3: *Comparison of Distribution Accuracy Between 4Cell® Nutri-T Media and Water*

Single-Use Assembly	Linkit® AX			
Bag volume on Linkit® AX [mL]	150	500	150	500
Number of trials performed	10	10	20	8
Targeted total weight in bags [g]	1,500	5,000	1,500	5,000
Approximated equivalent volume in bags [mL]	150	500	150	500
Total target weight [g]	1,750	5,250	1,580	5,250
Solution	4Cell® Nutri-T		Water	
Temperature [°C]	Ambient (22 ± 3)			
Distribution accuracy [%]	± 5.8	± 3.9	± 6.5	± 5.1

Trials with Different Total Target Weights

The distribution accuracy of the Linkit® AX was validated for different total target weights. The results are shown in Table 4.

The worst distribution accuracy performances were obtained with the lowest targeted total weight in bags (1,500 g), corresponding to 150 mL of water in each bag (± 6.5%). This can be explained by the fact that this is the current limit of the Linkit® AX solution. The total target weight of 1,580 g was a small value compared to the rest of the validated range; the pump has to start and stop right almost immediately, resulting in less accurate results.

The same distribution accuracy was obtained for the targeted total weight in bags of 5,000 and 10,000 g, corresponding to 500 and 1,000 mL of water in each bag (± 5.1%), respectively. For a targeted total weight in bags of 2,500 g, corresponding to 250 mL of water in each bag, better distribution accuracy results were obtained (± 4.0%) in comparison to the other values.

Impact of Performing Filtration Between the Pump and Linkit® AX Single-Use Assembly

The distribution accuracy of the Linkit® AX was compared in operations performed with and without a filter. The results are shown in Table 5.

Our findings show that filling speed was the most significant factor impacting filling accuracy. The higher the filling speed, the smaller the weight standard deviation and, therefore, the better the distribution accuracy between the bags. As such, the filling speed is key to ensure that the flow rate before the filter imposed by the pump remains the same at the filter outlet. This will ensure that the flow rate is the same at the main inlet of Linkit® AX single-use assembly, preserving accuracy similar to that without a filter.

Table 4: *Comparison of Distribution Accuracy With Different Total Target Weight Values*

Single-Use Assembly	Linkit® AX			
Bag volume on Linkit® AX [mL]	500	500	500	1,000
Number of trials performed	20	20	8	60
Targeted total weight in bags [g]	1,500	2,500	5,000	10,000
Approximated equivalent volume in bags [mL]	150	250	500	1,000
Solution	Water			
Temperature [°C]	Ambient (22 ± 3)			
Distribution accuracy [%]	± 6.5	± 4.0	± 5.1	± 5.1

This conclusion is confirmed in Table 5, which shows the distribution results of the same configuration as in the validation but with a filter between the pump and Linkit® AX main inlet line. The filter was chosen to maintain the same flow rate as without a filter at the inlet of Linkit® AX. The distribution accuracy obtained was ± 5.4%, compared to ± 5.1% without a filter. If the flow rate at the outlet of the filter and so at the inlet of Linkit® AX is lower, it will have the same negative impact on filling accuracy as if the filling speed is lower.

Table 5: *: Comparison of Distribution Accuracy Between Operations With and Without a Filter*

Single-Use Assembly	Linkit® AX	
Number of trials performed	8	62
Bag volume on Linkit® AX [mL]	1,000	
Targeted total weight in bags [g]	10,000	
Approximated equivalent volume in bags [mL]	1,000	
Total target weight [g]	10,250	
Solution	30% wt sugar	Water
Temperature	Ambient (22 ± 3)	
Filter	Yes	No
Distribution accuracy [%]	± 5.4	± 5.1

Conclusion

No significant difference was observed between 4Cell® Nutri-T media aliquoting operations and water operations in the trials performed. For the trials with different total target weights in bags, the worst results obtained were with 1,500 g (equivalent to 150 mL of water in each bag). This corresponds to the minimum total target weight with which Linkit® AX can be used. Below this volume, the accuracy performances are severely impacted.

For the other total target weights in bags, 2,500, 5,000, and 10,000 g (corresponding to 250, 500, and 1,000 mL of water in each bag, respectively), the distribution accuracy is ± 4.1%, ± 5.1%, and ± 5.1%, respectively.

The impact of a filter between the pump and Linkit® AX is negligible if the filter has been chosen to ensure the same flow rate is kept at the inlet and outlet of the filter.

Overall Conclusion

The DoE performed led to the following conclusions:

1. The factors with a significant impact on distribution and filling accuracy are:
- Filling pump speed (the flow rate at the inlet of Linkit® AX single-use assembly). The higher, the more accurate the filling.
- Total target weight. The higher, the more accurate the filling.

2. The factors without a significant impact on distribution and filling accuracy are:
- The number of bags filled
- The solution used for aliquoting (viscosity)

3. The optimized process parameters for best accuracy are:
- For total weight to be filled in bags ≥ 5,000 g (corresponding to 500 mL of water in each of the ten bags):
  - Air purging speed: 50 rpm
  - Filling speed: 250 rpm
  - Slow down at: 99% full
  - To final speed: 150 rpm

- For total weight to be filled in bags between 1,500 and < 5,000 g (corresponding to water volumes between 150 and 250 mL in each of the ten bags):
  - Air purging speed: 50 rpm
  - Filling speed: 250 rpm
  - Slow down at: 80% full
  - To final speed: 150 rpm

The distribution accuracy obtained with 4Cell® Nutri-T media, different total target weight values, and a filter in between the pump and the Linkit® AX single-use assembly led to the following conclusions:

- No significant difference was observed between 4Cell® Nutri-T media and water aliquoting operations.
- The worst results were obtained with a total weight in bags of 1,500 g (corresponding to 150 mL of water in each bag) with water and 4Cell® Nutri-T, confirming the validation performed. Comparable results were obtained with the other volumes.
- Similar results were obtained with or without a filter between the pump and the Linkit® AX single-use assembly. Including a filter does not affect accuracy as long as the flow rate at the main inlet line of Linkit® AX is maintained.

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