

This article presents a procedure for the validation of biological API manufacturing processes. It details each step of the procedure for biological processes applications, and covers the regulatory requirements for biological APIs.

Procedure for the Validation of Biological Active Pharmaceutical Ingredients (APIs) Manufacturing Processes

by Josée Ethier

Introduction

The validation of biological Active Pharmaceutical Ingredients (APIs) manufacturing processes is more complex compared to standard chemical APIs, due to the lack of both product and process characterization. The following definition of Process Validation can be found in ICH Guideline Q7A:¹ "Process validation is the documented evidence that the process, operated within established parameters, can perform effectively and reproducibly to produce an intermediate or API meeting its pre-defined specifications and quality attributes."

The objective of process validation is to:

- demonstrate process stability and reliability
- evaluate the impact of a variation in a critical process parameter on product quality

Not all manufacturing processes require validation. The examples mentioned hereafter usually require process validation:

- processes for which the product can not be fully characterized and/or verified
- processes for which the complete product characterization is very expensive or impossible to perform (in this case, product quality is assessed using process validation)
- processes for which the product quality is critical and for which a slight variation in its composition may result in severe reactions in the patient

Validation of processes involving micro-organisms probably present the highest difficulty degree due to the important variability of living organisms. Variations in the behavior and productivity level of the micro-organism used may result in differences in the composition of the culture media at the end of the production, which can, in turn, impact the purification process. Processes involving living micro-organisms are more sensitive to operating conditions and may show larger variability from one batch to another. Therefore, biological processes are more difficult to validate, and require more considerations. In addition, as stated by Kirrstetter,² the raw materials used in biological APIs manufacturing processes may result in microbiological contamination, and more stringent controls of equipment, utilities, and services are required to minimize the risk of contamination. Slight variations of the manufacturing conditions may be observed when undesired un-

Table A. Recommended procedure for the validation of API manufacturing processes.

Recommended Procedure for the Validation of API Manufacturing Processes
<ul style="list-style-type: none"> • Formation of a Validation Committee • Description of the process manufacturing steps, utilities, services and equipment • Critical Analysis • Identification of critical process parameters and validation requirements • Elaboration of a Validation Master Plan (VMP) • Redaction of process validation protocols • Protocols execution • Reports

characterized substances are found in the final product. Biological APIs are difficult to characterize and, are influenced by the numerous parameters involved in their manufacturing process.

As the biotechnology sector faces important growth with the increasing number of expiry patents,³ the number of biological API manufacturing processes to validate will increase in the next few years, enhancing the importance of a structured procedure for process validation.

Process Validation Requirements

Process validation is generally required prior to product commercialization to demonstrate that the process consistently results in a product having the required specifications

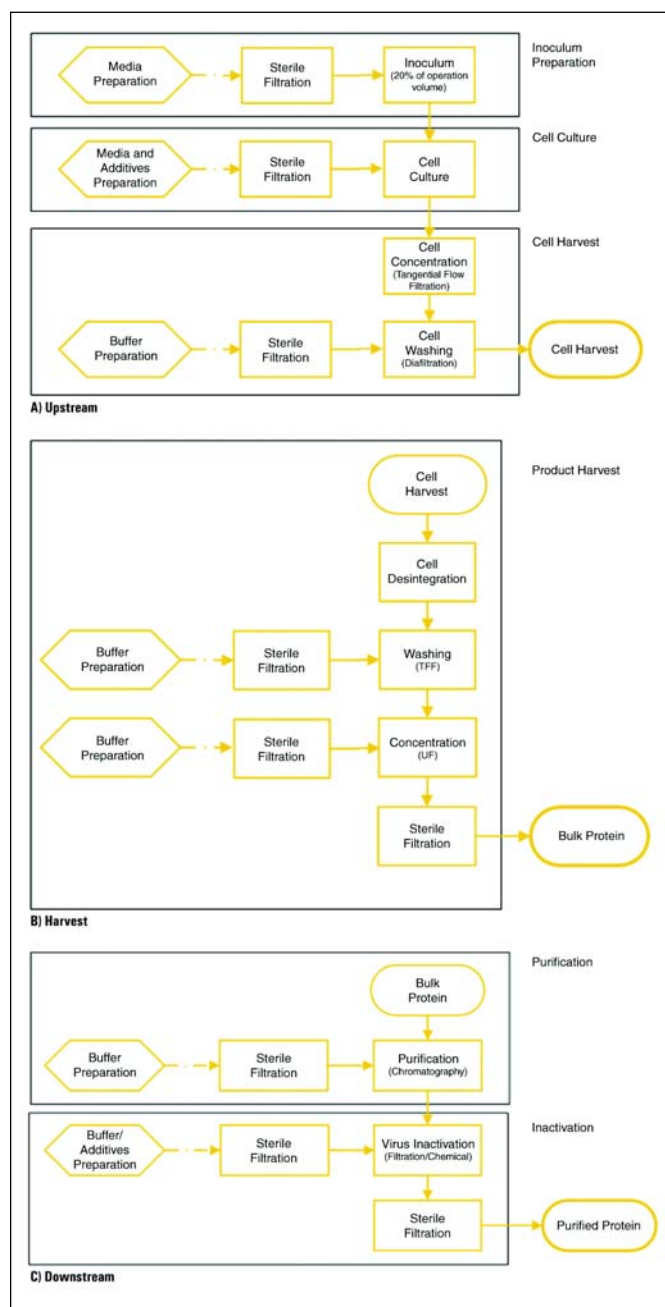


Figure 1. Typical flowchart for a cell culture process (and functional blocks).

and quality characteristics. Process validation was originally not required for clinical trial material manufacturing due to the numerous modifications brought to the procedures during process fine tuning and due to the limited number of batches produced at this stage.

The Annex 13 of European current Good Manufacturing Practices⁴ has been modified in 2003 to require process validation for clinical material manufacturing.

The Canadian regulatory agency has decided to adhere to the new European directive.⁵ FDA still maintains its position and requires process validation for commercial batches only. However, the US FDA appropriated control and monitoring of the critical process parameters and demonstrated traceability for clinical lots.

Process validation will now be required earlier, prior to the completion of process development. The validation procedure presented here constitutes a systematic approach that also can help in process development and optimization, and allow the initiation of process validation while its development is still in progress.

Main Validation Steps

The proposed validation procedure for biological API manufacturing processes is described here. The procedure also can be applied to standard (non-biological) APIs.

Process validation may follow Installation, Operation, and Performance Qualification of equipment, utilities, and services, as a process can be developed and implemented in an existing premise using qualified equipment. However, the procedure presented here is more general and can be applied to new processes implemented in new premises using new services and equipment. As mentioned above, the proposed procedure can be initiated in early development stages and help in the identification of critical process parameters and improve process understanding. It also ensures that an appropriate distance has been taken to visualize the process as a whole and that equipment, utilities, services, process environment, and parameters impact have been properly considered and evaluated.

The main validation steps are described below and summarized in Table A.

Formation of a Validation Committee

First, a validation committee should be formed and include people from the following departments: Quality Assurance, Engineering, Production, and Development (R&D). Representatives from additional departments could be included if required (Laboratories, Logistics, and Regulatory Affairs, as an example), depending on the nature of the process to be validated and the organizational structure. This committee is responsible for the management and the execution of process validation and to ensure compliance with regulatory authorities. The committee should meet on a regular basis as long as process validation is not completed and be involved earlier during process development.

The modified Annex 13 to the European cGMPs⁴ requires the presence of a Qualified Person (QP) that plays a similar role.

Process Description

Once the committee is formed, a complete and detailed description of the manufacturing process can be established and should include the following steps:

1. A Process Flow Diagram (PFD) should be prepared and functional blocks should be defined, each block having a clear and distinct function. Figure 1 illustrates a typical flow chart of a cell culture process and the corresponding suggested functional blocks. Typical unit operations involved in a biological API manufacturing process are: fermentation, inoculation, cell harvest, filtration, centrifugation, diafiltration, chromatography, formulation, filling, freeze drying, sterilization. Transfer steps should be included in the PFD.
2. The equipment, utilities, and systems required can be listed for each functional block. The corresponding specifications and validation status (if any) should be specified.
3. The In-Process Controls (IPC) with the corresponding tolerances should be identified.
4. Product final specifications with the corresponding tolerances should be identified.
5. The analytical methods, required instruments, and final specifications with tolerances should be specified. The corresponding validation status (if any) should be specified.
6. The process parameters which impact product quality should be identified.
7. Process inlets and outlets should be identified and illustrated using a cause and effect diagram ("Fish Bone," see Figure 2). The "Fish Bone" illustrates the impact of a variation in process parameters on product specifications and helps in process understanding.

Critical Analysis

The validation of a Drug Product (DP) manufacturing process requires the qualification of each manufacturing step whereas the validation of an API manufacturing process requires the qualification of the critical manufacturing steps only.

To help in the identification of validation requirements and critical process parameters (and complete the lists prepared during the previous step) a Critical Analysis (or Risk Analysis) needs to be performed - *Table B*. Most product nonconformities result from either errors performed during manufacturing or from variations in process parameters or immediate environment.

A Critical Analysis consists of the identification of those possible sources of errors and process variations that could result in product non-conformity. Once the sources are identified, their impact on product quality and/or process safety (including environment and operators) are evaluated. The

Critical Analysis Results
<ul style="list-style-type: none"> • Identification of the critical process parameters that must be monitored and controlled. • Identification of validation requirements: IQ, OQ, PQ, analytical methods, cleaning, operation, inactivation, process • Identification of the required characteristics: raw materials, final product, in-process samples, etc. • Identification of "markers" for process comprehension and follow-up.

Table B. Description of the critical analysis results.

probability of detection (D), the possible occurrence of the problem (O), and the gravity of the resulting consequence (G) are evaluated (refer to the Failure Mode and Effect Analysis procedure).⁶ The critical parameter ($C = D \cdot O \cdot G$) can then be calculated, and represents a quantitative measure of the critical of each possible source of non-conformity. Appropriated solutions can be proposed for each possible source identified to reduce the critical (C) to an acceptable level, either by improving the detectability of the problem (and reduce factor D) and/or reducing the possible occurrence of the problem (and reduce factor O). The calculation of the Critical Factor (C) can be performed again, considering the solutions proposed.

Most of the time, the solutions proposed will allow the identification of critical process parameters and the definition of validation requirements: perform cleaning or design validation, add an in-process control, control the environment (by having a validated HVAC system as an example), etc. Table C illustrates an example of a part of a Critical Analysis (inoculation functional block). Four possible sources of nonconformities have been identified to illustrate the procedure (not exhaustive):

- the inoculum cell concentration could be out of specification
- the inoculum could be contaminated
- the transfer of the inoculum to the bioreactor could be deficient
- the bioreactor cleaning could be deficient

The impact of each source is then identified and evaluated. An out of specification inoculum cell concentration would result in unusual growth kinetics that could be detected using an in-process control prior to bioreactor inoculation. Accordingly, the possibility of detection (D) would be rated at 3, the occurrence (O) at 2, and the gravity (G) at 3 since the productivity level would be affected. The resulting critical factor (C) would then be 18 ($3 \cdot 2 \cdot 3$). The proposed corrective action is the implementation of optical density verification prior to inoculation. The probability of detection (D) would then be rated at 1, the occurrence (O) at 1, resulting in a critical factor (C) of 3 ($1 \cdot 1 \cdot 3$).

The final critical factor evaluation (rightmost column in Table C) allows the identification of most critical process parameters with a series of corrective actions that are required to keep the risks at a minimal level and ensure product consistency and quality, in addition to personnel safety and

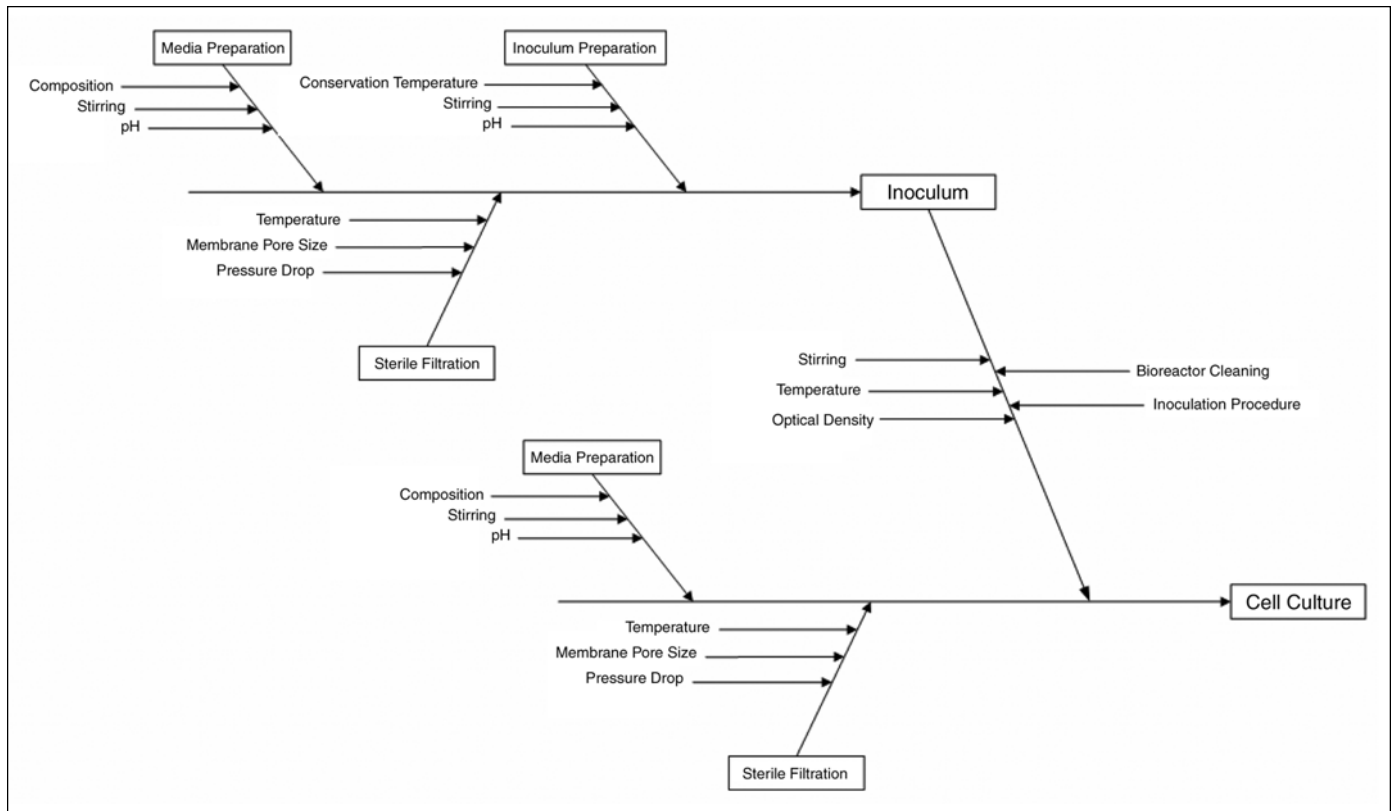


Figure 2. Example of a cause and effect diagram ("Fish Bone.")

environmental considerations. The corrective actions could be an in-process control, a standard operating procedure, a validation, a verification, personnel training, or any other action required to reduce the occurrence of the problem and/or improve its detectability.

Generally, the following process steps should be identified as critical for biological processes and be included in the Critical Analysis: equipment and instrument cleaning, raw material characterization (media, components, and cells), weighing, solution, and media preparation, inoculation preparation, bioreaction (pH, dissolved oxygen, stirring,...), harvesting, refolding (if required), purification, formulation (when applicable), filling, freeze-drying (when applicable), packag-

ing and labelling.

Identification of Critical Process Parameters and Validation Requirements

The Critical Analysis is a very efficient tool that allows the identification of critical process parameters and the identification of validation requirements. Any possible source of non conformity is analyzed and solutions to control variability are proposed until the critical factor (C) is reduced to its minimal value. The acceptable operation range for each critical process parameter also can be defined. The risk to forget an important parameter or to omit a required qualification is reduced to its minimum, and benefits are reflected on the

Manufacturing Step	Source of Non-Conformity	Impact on Quality/Safety	D	O	G	C	Corrective Action	D	O	G	C
Bioreactor Inoculation	Out of specification cell concentration of the inoculum	Unusual growth kinetics	3	2	3	18	- In-process control of optical density before inoculation	1	1	3	3
	Contamination while inoculation	Presence of contaminant in the bioreactor	3	2	4	24	- SOP for inoculation - Installation of a laminar flow hood - SIP of inlet ports to be developed - Monitor optical density during growth phase	1	1	4	4
	Forget to open inlet port	Loss of inoculum	1	2	3	6	- SOP for inoculation	1	1	3	3
	Bioreactor cleaning deficient	Presence of contaminant in the bioreactor	3	2	4	24	- Cleaning validation required - TOC/swab prior to bioreactor inoculation	1	1	4	4
LEGEND			D = probability of detection		O = possible occurrence of the problem		G = gravity of the resulting consequence		C = critical factor		

Table C. Example of a critical analysis.

whole validation team, for which the validation/development objectives are clear even before the redaction of the Validation Master Plan. Some corrective actions could involve process development and/or engineering design.

Some “markers” can be chosen among the critical process parameters identified, based on their fair representation of the process condition, to limit in-process control related costs during process validation and commercial manufacturing (methods development and validation, as well as time and consumables). The optimal value of each marker should be identified and accompanied with the corresponding analytical method, sampling procedure, specification and tolerance, and a reference standard (if required). Markers are used mainly for not-well characterized biologics (mainly complex vaccines, blood products, viral vectors, and cell therapies)⁷ to follow the product quality and consistency.

Of all unit operations, cell culture is the processing step resulting in most important variations. Living micro-organisms can show slight differences in growth characteristics and can release proteins and debris in the culture media that affect the growth curve. The Critical Analysis is therefore of major importance for biological processes validation.

Bioassays also are critical and should be qualified since they allow the determination of the product’s tertiary structure and activity.⁷

Validation Master Plan

Now that the critical process parameters, critical manufacturing steps, markers, and validation requirements are identified, the preparation of a Validation Master Plan should constitute the normal following step of the process validation procedure.

For biological processes, cell stability and purity (including viral clearance) also need to be validated. The stability of the genetically modified micro-organism must be demonstrated, and the maximum cell division number identified. This can determine the longest continuous cell culture that could be performed before mutation or transformation occurs. Viral clearance is usually performed by inactivation (using pH, solvents and/or detergents, or heat) or removal (using filtration and/or chromatography).

For yeast and bacterial cultures, viral inactivation is not required since these micro-organisms are usually not in contact with viruses nor TSE (Transmissible Spongiform Encephalopathies).

Process Validation Protocol Redaction

The structure of process validation protocol should be the same as for standard pharmaceutical processes. However, for biological processes, particular verifications are required to ensure process consistency and reproducibility.

The process validation protocols should include:

- a general decisional process flow chart
- a list of the equipment (both critical and ancillary) and instruments used, including their identification number and calibration state (if required)

List of Biological Process Validation Pre-Requisites
<ul style="list-style-type: none"> • Defined raw materials • Defined equipment, utilities and services • Defined process parameters and acceptance range • Standard Operating Procedures (SOPs) • Process documentation • Critical analysis (risk analysis) • Validation Master Plan • IQ/OQ/PQ for equipment, utilities and services with closed deviations and nonconformities • Validated analytical methods • Qualified instruments • Maintenance and calibration programs • Change control program • Identification of critical process parameters and markers, with their respective acceptance range • Bioassays defined and qualified

Table D. List of the information required prior to process validation.

- a description of all manufacturing steps and in-process controls, including packaging operations and acceptance limits for each process parameter
- the final product specifications

The following verifications should be included for a biological API manufacturing process:

Bioreaction

As mentioned above, bioreaction is the most variable manufacturing step as micro-organisms are involved.⁷ The process duration, temperature, pH, conductivity, nutrients, and product concentration should be characterized. The establishment and maintenance of the Working Cell Bank (WCB) is not covered by the ICH Q7A cGMP. However, it is recommended to perform the characterization of the Master and Working Cell Bank (MCB/WCB) to identify the history of the organism (media used, storage conditions, pressure factor used, etc.) and demonstrate the absence of virus and mycoplasma.

Growth kinetics, product formation rate, yields, cell density, stirring conditions, and optical density needs to be characterized for successful process scale-up and validation. The demonstration that the bioreactor sterility can be maintained during normal operation of the bioreactor (sampling, addition of antibiotics, etc.) should be included in the performance qualification.

Cleaning procedures are critical for bioreactors as they must eliminate any risk of cross contamination. It is strongly recommended to dedicate bioreactors to bacteria, yeast, or mammalian cell culture to avoid contamination.

Continuous operation of a bioreactor (including perfusion) is often preferred to batch and fed-batch operation for mammalian cell culture due to higher product yields obtained. However, validation is more difficult to perform on continuous systems since the production can last for months (compared to days/weeks for batch and fed-batch cultures). The cell line stability can be difficult to demonstrate. Cell mutation can occur and can be difficult to detect, resulting in complex validation. It may be difficult to determine when a lot begins and finishes, and the production of three distinct

batches can take a year to perform. In addition, if a contamination occurs, it will be detected only few days later, contaminating part of the lot.

Finally, it is important to characterize product stability for the holding period between bioreaction and harvest. The maximum holding duration and storage conditions need to be specified.

Harvest and Recovery

Most unit operations used in harvest and recovery steps are centrifugation, microfiltration (tangential, diafiltration), disintegration, and refolding (for inclusion bodies). At the end of the recovery step, the product is most of the time inactivated using sterile filtration to eliminate any risk of contamination of the equipment used for downstream processing (mainly chromatography media) and to contain viable micro-organisms into the production room.

The efficiency of each filtration step should be characterized and verified. The filter integrity should be demonstrated before and after filter use using standard methods. Filter integrity also should be demonstrated before and after the sterile filtration step. Sterile filtration qualification should include the demonstration of absence of viable particles following filtration.

The acceptance range of each critical parameter needs to be specified for each unit operation involved in harvest and recovery operations. Refolding step should be defined and its consistency demonstrated.

The endotoxin and protein concentration shall be characterized at the end of the recovery step. And as previously specified, the hold period between recovery and purification needs to be characterized (maximal duration and storage conditions) to ensure product integrity and stability.

Downstream Processing

Unit operations usually involved in downstream processing are chromatography (gel filtration, ion exchange, affinity, hydrophobic interaction), extraction, and ultrafiltration. Five to 10 purification steps are normally required to reach the required product purity level.

Chromatography requires several verifications to demonstrate process consistency and reproducibility and to guarantee product quality. Chromatography media is very difficult to clean, and is often used for different applications. Cleaning validation is critical for chromatography columns and media. The Total Organic Carbon (TOC) of the final rinsing water should be measured and be kept below the specification to demonstrate the absence of residues of cleaning agents and confirm the absence of resin leaching. The gel lifetime also should be determined. A procedure is required for column sanitization and needs to be rigorously followed by the personnel.

Chromatography resins should be considered as a raw material and therefore requires full characterization as well as acceptance specifications. Resins properties can vary considerably from one batch to another and the acceptance specification range should consider such possible variability.

Purification efficiency is closely related to chromatography operation parameters such as ionic strength, pH and flow rate of the elution solution, the column diameter, the bed height, and both the impurity and protein concentration of the inlet solution to be purified. The efficiency is also dependant on packing quality. The evaluation of the Height Equivalent to a Theoretical Plate (HETP) should be performed following each column packing to demonstrate the absence of channeling and assess packing quality. Finally, the non-specific binding of the protein to the chromatography resin should be quantified to confirm resin quality and safely process the target protein using this media.

For ultrafiltration operations, the maximum flow rate and membrane pore size should be characterized and an operating range specified. The non-specific binding of the protein on the membrane should be quantified as for chromatography resins.

The use of disposable filtration units eases the validation of filtration steps and reduces cleaning validation efforts.

Validation Execution

ICH¹ requires three consecutive runs for prospective and concurrent process validation and from 10 to 30 consecutive runs for retrospective process validation. More runs may be required for complex processes. For prospective and concurrent validation, three different lots of raw materials should be used. Each run needs to be completely independent from the others: the inoculum and culture media should be fresh and the equipment cleaned using cleaning procedures in place between each run.

The application of the validation procedure described here ensures that all the information required to perform process validation is available - *Table D*.

Product characterization can be performed using the specified analytical methods. Data needs to be compiled and statistically analyzed.

Finally, a validation report comparing the product specifications with product characterization should be prepared. Deviations and nonconformities should be summarized in this report.

A change control procedure is required to follow and control any modification performed on the process and/or its utilities and services. Each modification should be evaluated commonly by the Validation Committee or by the Qualified Person, depending on the structure of the society. Process revalidation may be required when a major modification is performed on an equipment, a service, or an utility, on the premise itself or on the manufacturing procedure. Any deviations of the markers identified should result in a process investigation to quickly identify the source of the problem and reduce the risk of getting out-of-specifications product.

Conclusion

The proposed procedure may seem time-consuming and heavy to implement. However, considerable reduction of both development and validation steps duration results from the use of this procedure. Qualification can be initiated prior to the

completion of process development and accelerate product marketing.

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