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A BRIEF REVIEW ON CLEANING VALIDATION AND ITS SIGNIFICANCE IN PHARMACEUTICAL INDUSTRY

P.V. Waghmare*, A.S.Chinchole[1], B.N.Poul[2], O.G.Bhusnure[3]

*Master of pharmacy, Department of Quality Assurance, Maharashtra College of Pharmacy, Nilanga, Dist. Latur (MS) 413521, India
[1]Master of pharmacy, Department of Quality Assurance, Maharashtra College of Pharmacy, Nilanga, Dist. Latur (MS) 413521, India
[2]Principal of Maharashtra College of Pharmacy, Nilanga, Maharashtra College of Pharmacy, Nilanga, Dist. Latur (MS) 413521, India
[3]Head of Department, Department of Quality Assurance, Maharashtra College of Pharmacy, Nilanga, Dist. Latur (MS) 413521, India

ABSTRACT

In the manufacturing of the pharmaceutical products it is a must to reproduce consistently the desired quality of product. The current Good Manufacturing Practice (cGMP) regulations recognize that cleaning is a critical issue to ensure product quality. The control of cross contamination plays a very important role in maintaining the quality of the product. cleaning in a given pharmaceutical production equipment to prevent cross contamination and adulteration of drug products with other active ingredients like unintended compounds or microbiological contamination, leads to prevent several serious problems and also useful in related studies like packaging component cleaning validation. The manufacturing of API and pharmaceutical products involves series of processing steps and use of various equipments. The benefits due to cleaning validation are compliance with federal regulations, identification and correction of potential problems, previously unsuspected which could compromise the safety and efficacy of drug products. This article provided background on cleaning validation and the associated regulations, cleaning methods, validation strategy, validation samples, acceptance criteria, clean hold time, training, change control, and revalidation. The documented evidence of the consistent performance of the cleaning process is given by the validation process. It ensures safety, efficacy, and quality of the subsequent batches of drug product. In this article the various aspects of the cleaning validation such as different types of contaminants, sampling procedures, analytical techniques and regulatory requirements are discussed in detail. In this article cleaning validation and its importance are also discussed in brief.

Keywords: Cleaning validation, Potential Residue, Levels of cleaning, Elements of cleaning validation, Analytical testing method.

INTRODUCTION

The cleaning validation is necessary to establish the consistency and uniformity by discussing practices that have been found acceptable. Cleaning validation is primarily applicable to the cleaning of process manufacturing equipment in the pharmaceutical

industry. The focus of cleaning validation is those cleaned surfaces that, if inadequately cleaned, could potentially contaminate the product subsequently manufactured in that same equipment. Cleaning validation (CV) is driven by regulatory expectations to ensure that residues from one product will not carry over and cross-contaminate the next product. Regulatory scrutiny is more rigorous in a multiproduct facility, compared with a single product establishment. Companies are usually cited, either for not having a sound cleaning validation, or not meeting the protocol acceptance criteria. Because failing protocol acceptance criteria is considered a substantial regulatory risk, companies are forced to spend money and resources, even though there is minimal, or no product risk.

The basic mechanisms involved in removing the residues and contaminants from the equipment are mechanical action, dissolution, detergency and chemical reaction. The important task of performing a cleaning validation is to identify and correction of potential problems previously unsuspected which shows effect on safety, efficacy, or quality of subsequent batches of drug product produced within the equipment.

It is vital for a successful cleaning validation to have appropriate acceptance criteria. In developing the acceptance criteria, companies may adopt a conservative approach, either to prove that they have a sound cleaning validation program, or to ensure that field data (results) will reflect the acceptance criteria. The Food and Drug Administration's (FDA) guidance for determining residue limits is, that they must be logical (based on understanding of the process), practical, achievable, and verifiable. In validation, adequacy of each cleaning procedure requires demonstration that it can reliably and effectively remove, or reduce residues to an acceptable level, such that residues from the production of one product will not carry over in significant amounts to the next product. Companies today are faced with the challenge of reducing validation costs in an environment that demands increased compliance with current good manufacturing practices (cGMP). One should recognize that with cleaning validation, as with validation of other processes, there can be more than one way to validate a process. At the end, the test of any validation process is whether scientific data shows that the system consistently does as expected and produces a result that consistently meets predetermined specifications.

DEFINITION:

Validation (drug manufacture) [11]:-

In the pharmaceutical, medical device, food, blood establishments, tissue establishments, and clinical trials industries, "validation is the documented act of demonstrating that a procedure, process, and activity will consistently lead to the expected results". **OR** "Validation is the collection and evaluation of data, from the process design stage throughout production, which establishes scientific evidence that a process is capable of consistently delivering quality products [12]."

It often includes the qualification of systems and equipment. It is a requirement for good manufacturing practices and other regulatory requirements. Since a wide variety of procedures, processes, and activities need to be validated, the field of validation is divided into a number of subtypes including the following:

A. **Cleaning validation**
B. Process validation
C. Analytical method validation
D. Computer system validation

CLEANING VALIDATION:

This validation is used to show proof that the cleaning system consistently performs as expected and provides scientific data that consistently meets pre-determined specifications for the residuals. The cleaning validation process must be written into protocols and standard operating procedures which are detailed and specific for the different pieces of equipment and instrumentation used by the facility for each type of drug product produced. Other protocols and SOP's are also required based on the type of product manufactured or process used (such as a batch or bulk process or shared versus dedicated equipment).

A final report on the cleaning validation system will attest that the studies and data prove that the process is in control and cleans as expected. This report will also detail when and why revalidation needs to take place.

OBJECTIVE:

The objective of the cleaning validation is to verify the effectiveness of the cleaning procedure for removal of product residues, degradation products, preservatives,

excipients, and/or Cleaning agents as well as the control of potential microbial contaminants. In addition one need to ensure there is no risk associated with cross contamination of active ingredients. Cleaning Procedures must strictly follow carefully established and validated methods. Therefore, it is Important to do a step-by-step evaluation of API process to determine the most practical and efficient way to monitor the effectiveness of the cleaning process.

It is necessary to Validate cleaning procedures for the following reasons:

- It is a customer requirement - it ensures the safety and purity of the product.
- It is a regulatory requirement in Active Pharmaceutical Ingredient product manufacture.
- It also assures from an internal control and compliance point of view the quality of the process.
- The equipment cleaning validation in an Active Pharmaceutical Ingredient (API) manufacturing and pharmaceutical production is necessary to prevent contamination of a future batch with the previous batch material [10].
- It demonstrates that the cleaning process can consistently remove residue of the subjected product below the established acceptance criteria [10].
- The main objective of cleaning validation of equipment / utensils / components is to demonstrate sufficient documented evidence to ensure that the cleaning process can consistently remove residue of the subjected product below the established Acceptance Criteria.

SCOPE:

This document on Cleaning Validation is intended to address special considerations and issues pertaining to validation of cleaning procedures for equipment used in the manufacture of pharmaceutical products, radiopharmaceuticals, and biological drugs. The document is also intended to establish inspection consistency and uniformity with respect to equipment cleaning procedures. Principles incorporated in international guidance have been taken into account in the preparation of this document. The document is intended to cover validation of equipment cleaning for the removal of contaminants associated with previous products, residues of cleaning agents as well as the control of potential microbial contaminants[32].

This Document will serve to:

1. Define the basic concepts and terms associated with Cleaning Validation in the Active Pharmaceutical Ingredient industry.
2. Serve as a guide from which Master plans, Protocols and Reports may be compiled.

TYPES OF CONTAMINATIONS: [8, 22, 23]

1. Cross contamination with active ingredients

Contamination of one batch of product with significant levels of residual active ingredients from a previous batch cannot be tolerated. In addition to the obvious problems posed by subjecting consumers or patients to unintended contaminants, potential clinically significant synergistic interactions between pharmacologically active chemicals are a real concern.

2. Contamination with unintended materials or compounds

While inert ingredients used in drug products are generally recognized as safe or have been shown to be safe for human consumption, the routine use, maintenance and cleaning of equipments provide the potential contamination with such items as equipment parts, lubricants, chemical cleaning agents and pieces of cleaning tools such as brushes and rags.

3. Microbiological contamination

There is chance of microbial growth if the processing equipment is not properly maintained, cleaned and stored. It includes preventive measures rather than removal of contamination[5]. Maintenance, cleaning and storage conditions may provide adventitious micro-organisms with the opportunity to proliferate within the processing equipment.

POTENTIAL RESIDUES [4]:

The Active Pharmaceutical Ingredient Industry involves (in general) the manufacture of Active Pharmaceutical Ingredients by both chemical and physical means through a Series of multiple step processes. Plants or individual pieces of equipment, including Ancillary equipment, may be used in multi-product manufacture or dedicated to individual products. The result of inadequate cleaning procedures is that any of a number of contaminants may be present in the next batch manufactured on the equipment such as:

1. Precursors to the Active Pharmaceutical Ingredient

2. By-products and/or degradation products of the Active Pharmaceutical Ingredient

3. The previous product.

4. Solvents and other materials employed during the manufacturing process.

5. Cleaning agents themselves and lubricants.

6. Micro-organisms:-This is particularly the case where microbial growth may be sustained by the product. Typical residual contaminants that can be important for cleaning validation studies include [9]:

a. Host-cell proteins
b. Lipids
c. DNA/host-cell nucleic acid
d. Endotoxins
e. Carbohydrates
f. Membrane/chromatography matrix leachable
g. Detergents
h. Viruses
i. TSEs
j. Mycoplasmas, bacteria, fungi.

Tables 1–3 list some of the most common agents BioReliance uses in these studies, but note that the final study design is developed with your specific process in mind. Studies typically involve 3–6 agents [9]

TABLE 1: COMMON BACTERIA AND FUNGI USED IN CLEANING VALIDATION STUDIES [9].

Species*	Gram reaction/cell morphology	Requirement	Bacteria or fungi	Lab assay time	Resistance to physical/chemical inactivation
Pseudomonas aeruginosa	Gram negative/rod	Obligate aerobe	Bacteria	3–14 days	Moderate
Candida albicans	Yeast	Facultative anaerobe	Fungi	5–14 days	Low
Aspergillus niger	Mold	Aerobe	Fungi	5–14 days	High

Clostridium sporogenes	Gram positive/spore forming rod	Obligate anaerobe	Bacteria	3–14 days	Moderate
Staphylococcus epidermidis	Gram positive/cocci	Facultative anaerobe	Bacteria	3–14 days	Low
Bacillus isolates	Gram positive/rod	Aerobe	Bacteria	3–14 days	High

TABLE 2: COMMON VIRUSES USED IN CLEANING VALIDATION STUDIES [9].

Virus*	Genome	Envelope	Family	Size (nm)	Lab assay time	Resistance to physical/chemical inactivation
Xenotropic murine leukemia virus (XMuLV)	RNA	Yes	Retro	80–110	7–9 days	Low
Murine minute virus (MMV)	DNA	No	Parvo	20–25	10–13 days	High
Porcine parvovirus (PPV)	DNA	No	Parvo	20–25	7–9 days	High
Pseudorabies virus (PRV)	DNA	Yes	Herpes	150–250	4–6 days	Medium
Bovine viral diarrhea virus (BVDV)	RNA	Yes	Flavi	40–70	7–9 days	Medium
Adenovirus (Adeno)	DNA	No	Adeno	70–90	12–14 days	High
Reovirus (Reo)	RNA	No	Reo	60–80	7–9 days	High
Hepatitis A virus (HAV)	RNA	No	Picorna	~30	18–21 days	High

TABLE 3: COMMON SPECIES OF MYCOPLASMAS USED IN CLEANING VALIDATION STUDIES [9].

A. laidlawii	M. orale
M. gallisepticum	M. pneumoniae
M. hyorhinis	M. synoviae

FDA REQUIREMENTS: [8, 13]

1. FDA expects firms to have written standard operating procedures (SOP) detailing the cleaning process used for various pieces of equipment.
2. If firms have a specific cleaning process for cleaning between different batches of the same product and use a different process for cleaning between product changes, FDA expects the written procedures to address these different scenarios.
3. If firms have one process for removing water-soluble residues and another process for non-water soluble residues, the written procedure should address both scenarios and make it clear when a given procedure is followed.
4. It is required by the FDA, in the general validation procedure, that the personnel responsible for performing and approving the study should comply with the acceptance criteria and the revalidation data.
5. FDA expects firms to prepare specific written validation protocols in advance for the studies to be performed on each manufacturing system or piece of equipment which should address such issues as sampling procedures, and analytical methods to be used including the sensitivity of those methods.
6. It is expected that firms conduct the validation studies in accordance with the protocols and document the result of studies.
7. Final validation report is to be approved by the regulatory board which states whether or not the cleaning process is valid.
8. FDA expects firms to have written general procedures on how cleaning processes will be validated [4, 13].
9. Besides assuring chemical cleanliness, "the microbiological aspects of equipment cleaning should be considered. This consists largely of preventive measures ..."
10. "Determine the specificity and sensitivity of the analytical method used to detect residuals or contaminants [4, 13]."
11. "The firm should challenge the analytical method in combination with the sampling method(s) used to show that contaminants can be recovered from the equipment surface and at what level [4] ..."
12. "Direct sampling (e.g., with swabs) is 'most desirable,' although rinse sampling may be satisfactory [4, 13]

LEVELS OF CLEANING [4]:

The degree or level of cleaning and validation required for processes in Active Pharmaceutical Ingredient manufacturing depends largely on:

A. The equipment usage (i.e. dedicated equipment or not)
B. The stage of manufacture (early, intermediate or final step)
C. The nature of the potential contaminants (toxicity, solubility etc.)

Each of the above three bullets must be evaluated based on the next product, not only toxicology etc. The rational for this statement is given below:

A. In general, the higher the potential for finished Active Pharmaceutical Ingredient contamination the greater the requirement to validate cleaning methods to ensure product safety.
B. Active Pharmaceutical Ingredient manufacturers may have different levels of cleaning requirements in facilities based on the stage of the process being cleaned and the subsequent product to be manufactured.
C. It is the responsibility of the manufacturer to demonstrate that the level of cleaning and validation performed is adequate based on each individual situation and on a justifiable scientific rational.
D. Cleaning should be carried out as soon as practical after the end of processing and should leave the plant in a suitable condition for next use.

Below table No.4 illustrates an example of how a company may decide on the level of Cleaning between lots [4, 5].

TABLE 4: LEVELS OF CLEANING

LEVEL	Thoroughness Of Cleaning	Cleaning Validation Required
LEVEL 2	i.e.1)Product changeover of equipment used in final step 2) Intermediates of one batch to final step of another. 3) Carryover of the previous product is critical.	Yes – essential
LEVEL 1	i.e.1)Intermediates or final Step of one product to intermediate of another 2) Early Step to intermediates in a product sequence. 3) Carryover of the previous product is less	Progression between level 0 and 2 depending on process and nature of contaminant based on scientific rational. (Lower acceptable carry over limits).

	critical	
LEVEL 0	i.e. 1) in-campaign, batch to batch changeover. 2) Only gross cleaning if carryover of the previous product is not critical.	No validation required.

NB: All Processes Must Be Evaluated Individually

Level 0 cleaning:

It is an in- campaign batch to batch changeover requiring no validation.

It can be of two types:

1. Done between intermediate steps in the same manufacturing process.

   Eg. Step B is performed immediately after Step A for the same product line.

2. Done between the steps in the manufacturing processes of 2 batches of the same product.

   Eg. For given equipment, Step A of the first batch is to be followed by the manufacturing of Step A of second batch.

Level 1 Cleaning:

This is used between manufacturing of different batches of the same product.

Example – In a manufacturing Campaign for product A, there are 3 Batches to be manufactured as shown below.

Batch 1 ⟹ Batch 2 ⟹ Batch 3

For a given equipment &/or equipment train, if batch 1 in the campaign is to be followed by batch 2 in the campaign, then a level 1 cleaning is required.

Level 2 Cleaning:

This is used between manufacturing of different Batches of different Product and / or at the end of manufacturing campaign even if same product is planned for the next campaign.

The above two degree or level of cleaning differs from each other in terms of the degree of risk associated with it, acceptance limit, and degree of cleaning & method of verifying the cleaning process, in below Table,

TABLE 5: COMPARISON BETWEEN LEVELS [10]

	Level 1	Level 2
Risk	Lowest	Highest
Acceptance Limit	Highest	Lowest
Degree of Cleaning	Less Extensive	More Extensive
Verification of Cleaning	Visual Inspection	Analytical Testing

TABLE 6: COMPARISON OVER LEVELS OF CLEANING [10]

Level	Attributes	Acceptance Criteria	Cleaning Validation
LEVEL 0	Batch to Batch cleaning in an Identical Process (Same intermediate & same API)	Visual Observation	Not required
LEVEL 1	Changeover between intermediate of one product to final intermediate of another product	General limit 500 ppm	Increases from not required to necessary
LEVEL 2	Changeover of one API to another API.	Based on TTD / Toxicity, 10 ppm whichever is lower	Essential
	Changeover of any intermediate to any API.		
	Changeover from early steps to final step of same product.		
	Change over from physical operation of one product to physical operation of neither product.		

ELEMENTS OF CLEANING VALIDATION [4]:

A brief outline of the various elements of a basic cleaning validation study is given below,

I. Establishment of acceptance criteria

II. Cleaning procedure

- Identification of the equipment

- characterization of the products (Previous: activity/toxicity, solubility, subsequent: dosage, lot size)
- determination and characterization of the cleaning agents

III. Analytical method and its validation

IV. Sampling Procedure and necessary validation of same.

V. Cleaning Validation protocol.

VI. Cleaning Validation report.

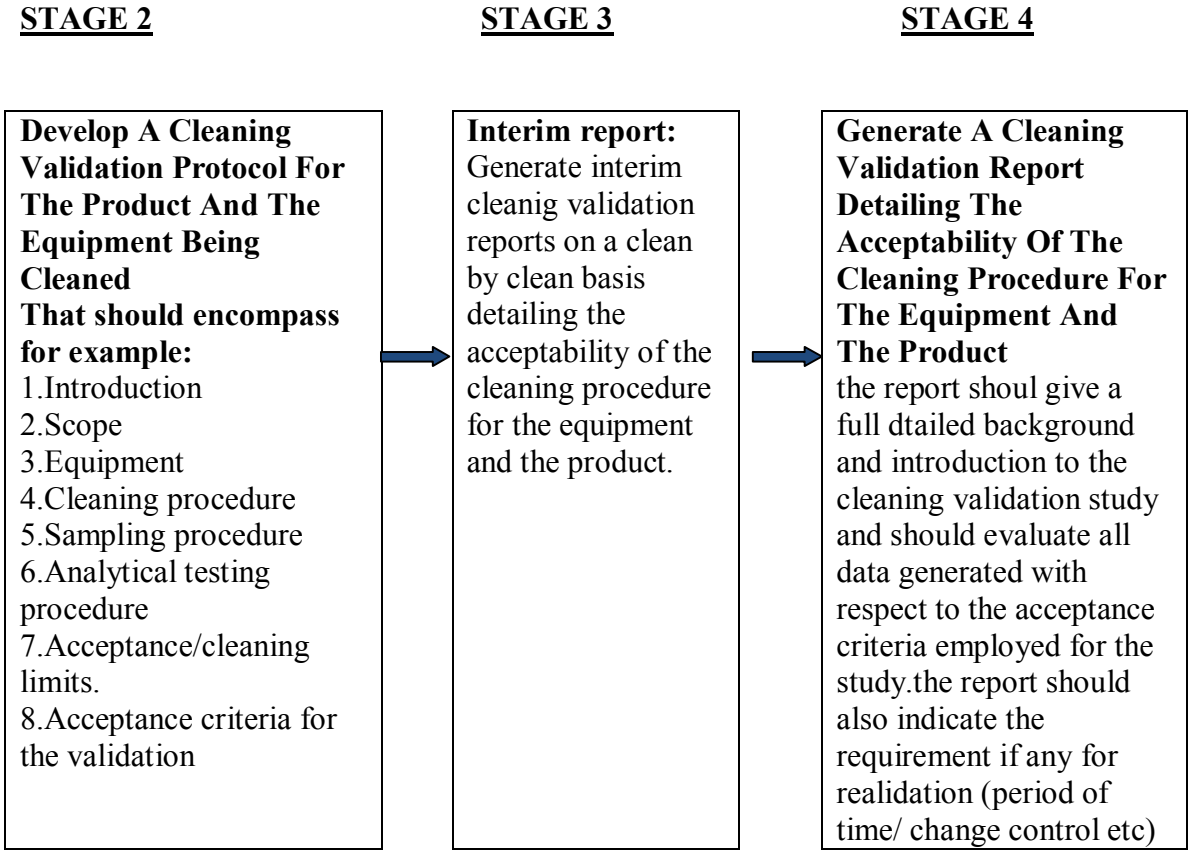
I. Establishment of Acceptance Criteria [2]:

The Cleaning Validation should demonstrate that the procedure consistently removes residues of the substance previously manufactured down to l0evels that are acceptable and that the cleaning procedure itself does not contribute unacceptable levels of residual materials to the equipment. The limits set should be practical, achievable and justifiable. In Active Pharmaceutical Ingredient manufacture there may be partial reactants and unwanted by-products which may not have been chemically identified. Therefore, it may be necessary to focus on by-products as well as the principle reactant. Companies should decide on which residue(s) to quantify based on sound scientific rational.

FIGURE 1: CLEANING VALIDATION PROCESS

STAGE 1

Determine The Most Appropriate Cleaning Procedure For The Equipment	**Development And Validation The Sampling And Chosen Analytical Methods For The Compounds Being Cleaned**	**Evaluate Equipment Surfaces And Determine**
1.Generate acceptance criteria data for the contaminant. 2.the cleaning method will be determined by the process, the equipment the cleaning agents the cleaning techniques available 3.All aspects of the cleaning procedure should be clearly defined in SOPs be they manual/CIP or COP.	1.Swab 2.Rinse (determine %recovery, Limit of detection, Limit of Quantitation, Accuracy of method, Reproducibility, Stability over time. Etc)	1.Worst case location to sample (swab sampling) 2.Volume of type of rinse solvent to be employed(rinse sampling) 3.Equipment surface area (necessary to calculate carryover into subsequent batches)



II. Cleaning Procedures [2, 3]:

Cleaning procedures should be sufficiently detailed to remove the possibility of any inconsistencies during the cleaning process. It is vital that the equipment design is evaluated in detail in conjunction with the product residues to be removed, the available cleaning agents and cleaning techniques when determining the optimum cleaning procedure for the equipment. Cleaning procedures should be sufficiently detailed to remove the possibility of any inconsistencies during the cleaning process.

A. Equipment parameters to be evaluated

- Identification of the equipment to be cleaned
- Difficult to clean areas
- Property of materials
- Ease of disassembly
- Fixed or not

B. Residues to be cleaned

- Cleaning limits
- Solubility's of the residues
- Length of campaigns

C. Cleaning agent parameters to be evaluated

- Preferably materials that are normally used in the process
- Detergents available (as a general guide, minimize use of detergents unless absolutely required)
- Solubility properties
- Environmental considerations.
- Health and safety considerations

D. Cleaning techniques to be evaluated

- Manual cleaning
- CIP (Clean-in place)
- COP (clean-out-of-place)
- Semi automatic
- Automatic
- Time considerations
- Number of cleaning cycles

E. Other requirements

III. (A) Analytical Method and Its Validation [3]:

The analytical methods used to detect residuals or contaminants should be specific for the substance or the class of substances to be assayed (e.g., product residue, detergent residue, and/or endotoxin) and be validated before the cleaning validation study is carried out. If levels of contamination or residual are not detected, it does not mean that there is no residual contaminant present after cleaning. It only means that the levels of contaminant greater than the sensitivity or detection limit of the analytical method are not present in the sample.

The basic requirements for the analytical method are as mentioned below:

1. The sensitivity of the method shall be appropriate to the calculated contamination limit.

2. The method shall be practical and rapid, and as much as possible, use instrumentation existing in the company.

3. The method shall be validated in accordance with the International Conference on Harmonization (ICH), the United States Pharmacopoeia (USP), and the European Pharmacopoeia (EP) requirements.

4. The analytical development shall include a recovery study to challenge the sampling and testing methods.

5. The ability to detect the target substance(s) at levels consistent with the acceptance criteria.[4]

6. The ability to detect the target substance(s) in the presence of other materials that may also be present in the sample (selectivity). [4]

7. Stability of samples over time if the time interval between removal and testing of samples potentially affects sample integrity. [4]

Various analytical techniques have been used for testing cleaning validation samples. Commonly used analytical tools for cleaning validation are mentioned in table-7.

TABLE 7: COMMONLY USED ANALYTICAL TOOLS FOR CLEANING VALIDATION [3]

Traditional Analytical Methods	Modern analytical Techniques
1.Gravimetry 2.pH 3.Conductivity 4.Colourimetry 5.UV-spectroscopy	1. Chromatographic techniques like HPTLC, HPLC and GC etc. 2.Total organic analysis(TOC) 3.Atomic absorption spectroscopy 4.Charged aerosol detection(CAD) 5.Immuno assay: ELISA 6. Capillary electrophoresis. 7.Optically simulated electron emission(OSEE) 8.Portable mass spectrophotometer 9.Bioluminescence

It includes both specific (e.g.HPLC) as well as non-specific methods (e.g.TOC, pH).Selection of suitable analytical method depends on various factors such as nature and type of analytes (Refer table-8)

TABLE 8: COMMONLY USED METHODS FOR SOME ANALYTES [3]

Analytes	Analytical method
Proteins	ELISA, HPLC, TOC
Organic compounds	TOC, HPLC, UV-VIS, TDS
Inorganic compounds	Conductivity, pH, TDS
Biological system	Vial cell analysis

III. (B) Analytical Method Validation [3, 7]:

Once the analytical method or technique of analysis has been finalized, the next step is validation of the method. The method validation includes checking the method for following parameters:

a. Precision, linearity, selectivity
b. Limit of Detection (LOD)
c. Limit of Quantitation (LOQ)
d. Recovery, by spiking

a) Linearity [7]:

Linearity refers to the characteristic of the relationship of the measured property to the level of analyte present. Linearity is an indication that the measured signal is directly proportional to the concentration of the analyte over the range. As a general rule for cleaning validation studies, the expectations are that assays will be linear over the range. Estimates of linearity can be made by such techniques as determination ~ (0.99 or better).

b) Accuracy [7]:

Accuracy refers to the trueness of the measurements to known values. This is determined by analyzing known standards. There is no "magic number" for acceptable accuracy. However, more accurate methods are preferred over less accurate methods. For example, if the acceptance criterion was 20 ppm a method with an accuracy of *2*- 10 percent, giving a result of 18 ppm could be considered an acceptable result. On the other hand, a method with an accuracy of *2*- 20 percent, giving a result of 18 ppm will be suspect in terms of meeting the acceptance criterion.

c) Precision [7]:

Precision refers to the reproducibility of the method and is often measured by standard deviation. Simple precision is the reproducibility of the results in the same lab over a series of replicate assays using the same operator, the same equipment, and usually on the same day. Intermediate precision is the reproducibility of results in the same lab using different operators, different pieces of equipment, and generally done on different days. Ruggedness is interlay reproducibility, involving reproducibility in different labs. The degree of accuracy required will depend on the specific situation. If the method is to be developed in a central lab and then transferred to several remote locations where analytical support for validation will occur, ruggedness should be evaluated. For a small start-up firm, the equipment and analysts may be limited, and simple reproducibility may be all that is required.

d) LOD / LOQ [7]:

LOD is the assay value at which it is still possible to say that the material is present, but it may be not possible to quantify with a specific value. LOD is typically estimated by several techniques. For example, for chromatographic techniques, LOD is estimated at three times the standard deviation of a baseline response. Values that are below the LOD are generally reported as < LOD. LOQ is the lowest assay value for which a reasonable confidence exists that the value is precise. There are also rules of thumb for estimating LOQs. For chromatographic procedures, the LOQ can be estimated as 10 times the standard deviation of the baseline noise. The LOQ can also be determined experimentally; as a practical matter, it can be considered the lower limit of the validated range of the assay. Any measured value below the LOQ is expressed as < LOQ.

IV. Sampling Procedure and Necessary Validation of same. (Sampling) [3, 4]

a) Sampling Locations, Surface area and number:

The hard to clean equipment locations (worst-case conditions) are identified based on cleaning experience and the design of equipment. Sample surface areas usually vary from 25 sq cm to 100 sq cm and should be large enough to allow the recovery of contamination quantity sufficient to be detected by the analytical method. The number of samples to be taken for the study depends on various factors such as the equipment surface area, construction material, design, shape and operating principle. Considering the homogeneity of the contaminant on the equipment product contact surface area,

several samples, but not less than three samples per piece of equipment, must be taken including the hardest to clean locations.

b) Sampling methods/techniques:

Sampling is the critical step in cleaning method validation. Different sampling methods/techniques have been used for cleaning method validation. The selection of either of these techniques must be consistent with sound scientific judgment and must support the objective of the study, which is to demonstrate that the amount of residual material in the equipment has been reduced to acceptable levels.

There are many types of sampling methods:

1. Swab sampling method (direct surface sampling):-This method is based on the physical removal of residue left on a piece of equipment after it has been cleaned and dried. A swab wetted with a solvent is rubbed over a previously determined sample surface area to remove any potential residue, and thereafter extracted into a known volume of solvent in which the contaminant active ingredient residue is soluble. The amount of contaminant per swab is then determined by an analytical method of adequate sensitivity. Swab sampling does not cover the entire equipment surface area therefore sites must be chosen with care. It is important those, as a minimum, the swab sites represent worst case locations on the equipment and that the result is then extrapolated to account for the total product contact surface area. This calculation makes it possible to make a worst case determination of potential carryover into subsequent product.

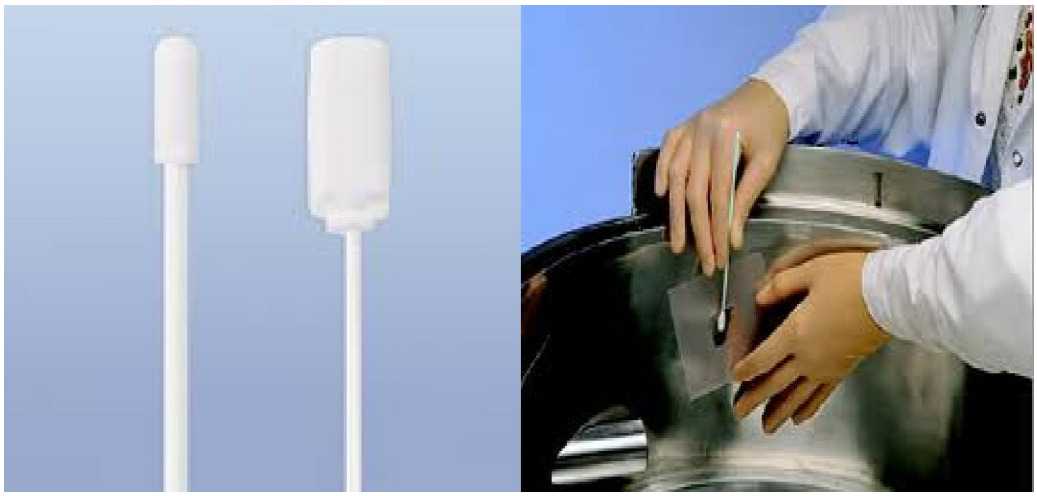


Figure 2: Swab sampling method

Due to the nature of this method which employs physical forces as well as chemical forces it may be necessary to perform sampling technique evaluation. A swab recovery study is performed to determine the ability of the swab to quantitatively remove the contaminant from the surface sampled. Generally, companies use special swabs available from suppliers such as: Whatman[R], Texwipe[R], or Coventry[R].

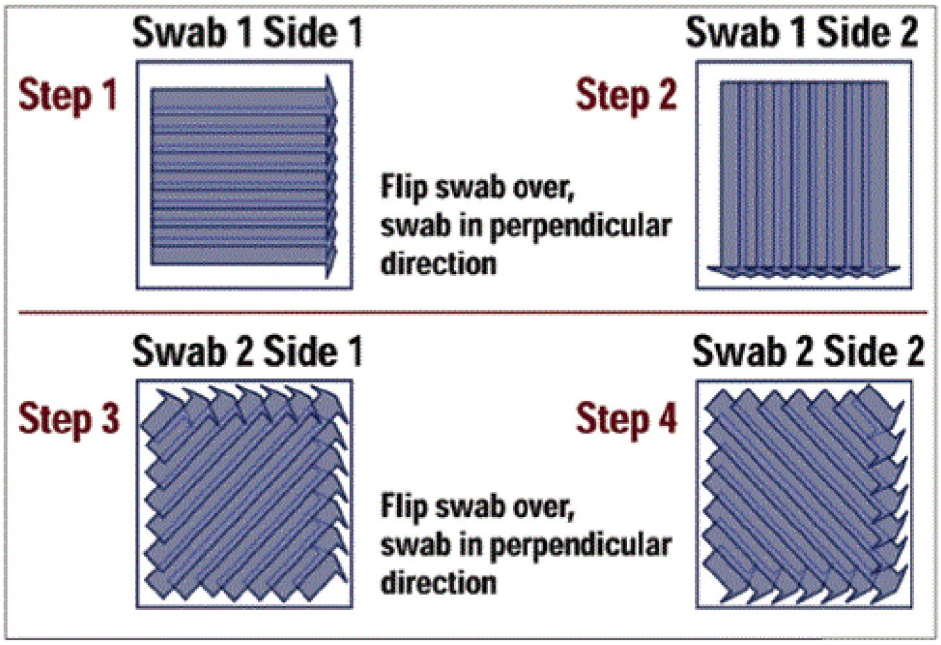


Figure 3: Recommended directions and motions of swabbing

Advantages [2]:

1. Dissolves and physically removes sample
2. Adaptable to a wide variety of surfaces
3. Economical and widely available
4. May allow sampling of a defined area
5. Applicable to active, microbial, and cleaning agent residues.

Limitations [2]:

1. An invasive technique that may introduce fibers
2. Results may be technique dependent
3. Swab material and design may inhibit recovery and specificity of the method
4. Evaluation of large, complex and hard to reach areas difficult (e.g., crevices, pipes, valves, large vessels)

2. Rinse sampling method:-This method is based on the analytical determination of a sample of the last rinsing solvent (generally water) used in the cleaning procedure. The volume of solvent used for the last rinse must be known to allow for the quantitative determination of the contamination. Thus, collection of rinse samples should consider location, timing, and volume. The solvent rinse occurs after cleaning has been completed. This method is important to ensure chosen solvent has appropriate recovery for residues being quantified [2]. This method allows much greater ease of sampling than swabbing. This method is not as direct as swabbing but will cover the entire surface area (and parts inaccessible to swabs).

Advantages [2]:

1. Adaptable to on-line monitoring
2. Easy to sample
3. Non-intrusive
4. Less technique dependent than swabs
5. Applicable for actives, cleaning agents and excipients
6. Allows sampling of a large surface area
7. Allows sampling of unique (e.g., porous) surfaces

Limitations [2]:

1. Limited information about actual surface cleanliness in some cases.
2. May lower test sensitivity.
3. Residues may not be homogeneously distributed.
4. Inability to detect location of residues.
5. Rinse volume is critical to ensure accurate interpretation of results.
6. Sampling methodology must be defined since rinse sampling method and location can influence results.
7. May be difficult to accurately define and control the areas sampled, therefore usually used for rinsing an entire piece of equipment, such as a vessel.
8. Reduced physical sampling of the surface.

3. Coupon sampling method:-In this method, coupons of the same materials of construction as the item to be cleaned can be affixed to the equipment, spiked with the

product, subjected to the cleaning procedures, and then submitted to the laboratory for direct analysis and recovery studies.

4. Solvent sampling method:-This technique uses a solvent not normally employed in the cleaning process to maximize recovery of expected residues. Known volume of solvent is applied to the surface in question. The method can be used in combination with swabbing.

5. Product sampling method:-This method is similar to placebo sampling except that it uses actual product. It requires examination of the next production batch for trace residuals of the previous batch.

6. Placebo sampling method:-It can be used to detect residues on equipment through the processing of a placebo batch subsequent to the cleaning process. Placebos are used primarily to demonstrate the lack of carryover to the next product. The placebo should mimic product attributes. The equipment characteristics also impact the choice of the placebo batch size.

Advantages [2]:

1. Placebo contacts the same surfaces as the product
2. Applicable for hard-to-reach surfaces
3. Requires no additional sampling steps

Limitations [2]:

1. Difficult to determine recovery (contaminants may not be evenly distributed in the placebo)
2. Lowers analytical specificity and inhibits detect ability
3. Takes longer and adds expense since equipment must be cleaned after the placebo run
4. Placebos must be appropriate for each potential product
5. Residues may not be homogenously distributed
6. No direct measurement of residues on product contact surfaces
7. The preferred sampling method and the one considered as the most acceptable be regulatory authorities is the swabbing method[10]

7. Direct sampling monitoring:-This method is used to evaluate surface cleanliness without surface contact, for example: measurement using spectrophotometric probes.

V. Cleaning Validation Protocol: [2, 4]

A Validation Protocol is necessary to define the specific items and activities that will constitute a cleaning validation study. It is advisable for companies to have drawn up a Master Validation plan indicating the overall Cleaning Validation strategy for the product range / equipment type / entire site**.** The protocol must be prepared prior to the initiation of the study and must either include or reference the documentation required to provide the following information:

1. **The objective of the study:**

What cleaning process is to be validated (indicating the product to be removed and the equipment from which it is to be removed)? If this study is to be employed to demonstrate the acceptability of the cleaning procedure for a group of products the rational for doing so should also be detailed here. The cleaning procedure(s) to be validated should be identified i.e. cleaning agents, soakage times, equipment parameters, number of cleaning cycles etc.

2. **Scope of the study:**

The company must evaluate the process and determine which residues are to be tested for and which are not to be based on sound scientific rational. What residues (including cleaning agents) are to be tested for, why those residues (if more residues may be present than are being tested for all residues should be under control see comments at 8.4). How many times the study should be run before a report is compiled and recommendations made.

3. **Listing of the process parameters to be verified:**

This is particularly necessary when automated or semi-automated cleaning techniques are to be employed.

4. **Sampling and inspection procedure to be used:**

The types of sampling methods to be used, where the samples are to be removed from and how many samples are to be taken. Any particular requirements should also be stated i.e. for sterile sampling / sampling light sensitive products. An equipment sampling diagram should be referenced.

5. **Personnel responsibilities during the study Test methods to be used:**

In order for the analytical testing of the cleaning validation samples (swabs or rinses) to yield meaningful results, the analytical methods used should be validated. This should be documented.

6. The basic requirements are:

The ability to detect the target substance(s) at levels consistent with the acceptance criteria. The ability to detect the target substance(s) in the presence of other materials that may also be present in the sample (selectivity) .The analytical method should include a calculation to convert the amount of residue detected in the sample to 100% if the recovery data generated indicates a recovery outside of an allowed range.

7. Change control:

Validated cleaning procedures should be included in the change control program. This will ensure that any proposed changes are evaluated fully for their impact on the validated state of the procedure. Where deemed necessary, the proposed revised procedure may need to be validated prior to routine implementation of Change control chapter in the CEFIC / EFPIA Guide entitled 'Good Manufacturing Practices for Active Ingredient Manufacturers' In the absence of an intentional change to a procedure, it is reasonable to assume that properly trained operators or a properly qualified automated system will be able to execute the procedure reproducibly and obtain the desired outcome - reduction of residue to acceptable levels [33].

VI. Cleaning Validation report [4]:

A validation report is necessary to present the results and conclusions and secure approval of the study. The report should include the following:

- Summary of or reference to the procedures used to clean, sample and test.
- Physical and analytical test results or references for same, as well as any pertinent observations.
- Conclusions regarding the acceptability of the results, and the status of the procedure(s) being validated.
- Any recommendations based on the results or relevant information obtained during the study including revalidation practices if applicable.
- Approval of conclusions.

- Review any deviations for the protocol that occurred.
- In cases where it is unlikely that further batches of the product will be manufactured for a period of time it is advisable to generate interim reports on a batch by batch basis until such time as the cleaning validation study has been completed. (Typically, in Active Pharmaceutical Ingredient Pharmaceutical manufacture, verification is deemed appropriate during development of the cleaning methods. Where products are manufactured infrequently, verification may be applied over a period of time until all measuring data has been collected for the Validation Report.)
- The report should conclude an appropriate level of verification subsequent to validation.

Validation report include (in equipment cleaning procedure):-

Equipment name:	
Calibrated/validated on:	
Location:	Revision no.:
Room no.:	Sampling technique:
Last product:	Cleaning sample analysis date:
B. no. of last prod:	Assay result
Book:	Test method reference:
Manufacturing date:	Ref. analytical log:
Active ingredient:	Limit of detection:
Therapeutic group:	Next product to be manufactured on same equipment:
Cleaning date:	Safety factor:
Cleaning SOP no:	

CHANGE CONTROL [21]:

Validated cleaning procedures should be included in the change control program. This will ensure that any proposed changes are evaluated fully for their impact on the validated state of the procedure. Where deemed necessary, the proposed revised procedure may need to be validated prior to routine implementation.

In the absence of an intentional change to a procedure, it is reasonable to assume that properly trained operators or a properly qualified automated system will be able to

execute the procedure reproducibly and obtain the desired outcome - reduction of residue to acceptable levels. There may exist special circumstances that would suggest that this assumption be verified via testing.

CONCLUSION

A wide range of factors influences the potential for cross contamination of materials, and the achievement of robust and effective cleaning operations offers a significant challenge to all product manufacturers. Cleaning validation is required in the pharmaceutical field to avoid potentially clinically significant synergistic interactions between pharmaceutical components. Cleaning validation there by assures the safety and purity of the finished products as well as avoiding cross contamination.

Therefore, an effective cleaning shall be in place to provide documented evidence that the cleaning methods employed within a facility consistently controls potential carryover of product including intermediates and impurities, cleaning agents and extraneous material into subsequent product to a level which is below predetermined level.

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REFERENCES

1. Syed IH, Syed EA: A Text Book of “Cleaning validation manual” A comprehensive guide for the pharmaceutical and biotechnology industries.
2. Kumar VS, Sanjeev T, and Sharma PK: “Overview of cleaning validation in pharmaceutical manufacturing unit”. International Journal of Advanced Research In Pharmaceutical & Biosciences, 2012; Vol.1 (2): 154-164.
3. Pawar HA, Banerjee ND, Pawar S, Pawar P: “Current Perspectives on Cleaning Validation in Pharmaceutical Industry: A Scientific and Risk Based Approach”.International Journal of Pharmaceutical and Phytopharmacological Research 2011, 1(1): 8-16.
4. ICH Good Manufacturing Practice Guideline for Cleaning Validation in Active Pharmaceutical Ingredients. (July 23 1999).

5. Harshavardhan K, Thiruvengada Rajan VS, Amruth Kumar N, Parameswari SA: "A review on role of cleaning validation protocol in Pharmaceutical manufacturing unit". International Journal of Review in Life Sciences, 1(3), 2011, 151-158.
6. Kumar S, Shashikant, Prashar B: "A review on concept of cleaning validation in pharmaceutical industry".International Research Journal of Pharmacy 2012, 3 (7).
7. Zahid Z and Rana Z: "Analytical Methods for Cleaning Validation". Scholars Research Library, Der Pharmacia Lettre, 2011, 3 (6): 232-239. (http://scholarsresearchlibrary.com/archive.html)
8. Lakshmana SP, Suriyaprakash TNK: "Cleaning Validation and its importance in Pharmaceutical Industry". Pharma Times - Vol 42 - No. 07 - July 2010.
9. Bioreliance by SAFC "A guide to planning your Cleaning Validation Study". Page no.1-12 (www.bioreliance.com)
10. Ghosh A, Dey S: "Overview of cleaning validation in Pharmaceutical industry".International Journal of Pharmaceutical Quality Assurance, 2010, 2(2):26-30.
11. Wikipedia free encyclopedia "Validation (drug manufacture)" (www.wikipedia.com)
12. FDA's Guidance for Industry, Process Validation: General Principles and Practices.
13. FDA, Guide to inspections of validation of cleaning process division of investigations, Office of regional operations & Office regulatory affairs. July 1993.
14. Pharmaceutical Inspection Convention, "Recommendations on Validation Master Plan, Installation and Operational Qualification, Non- Sterile Process Validation and Cleaning Validation", 2007.
15. Sharma PP: A Text Book of "Validation in pharmaceutical industry" (concept, approaches & guidelines) 2007, 1, 235-275.
16. Sharma PP, How to practice GMPs, A Guide for CGMP Compliance along with PAT & HACCP, 2004, 5, 28-35.

17. US Food and Drug Administration Guide to inspections of validation of cleaning processes. Rockville. MD. 1993.
18. "Cleaning Validation," 1999 Institute of Quality Assurance, Pharmaceutical Group No. 10.
19. Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme. Validation master plan installation and operational qualification: Non-sterile process validation. Cleaning validation. Geneva, Switzerland; 2004.
20. Sharma PP: A text book in Practice of Good Manufacturing Practices, third edition, Vandana publications, page no.99-105,108-109.
21. CEFIC / EFPIA Guide entitled 'Good Manufacturing Practices for Active Ingredient Manufacturers'.
22. APIC: Guidance on aspects of cleaning validation in active pharmaceutical ingredient plants; 2000; 16-46
23. APIC: Cleaning validation inactive Pharmaceutical ingredient manufacturing plants, 1999, 3-7
24. FDA. 1998. Human drug cGMP notes, vol. 6, no. 4. Rockville, Md., USA: Food and Drug Administration, Center for Drug Evaluation and Research.
25. Andy Walsh, Cleaning Validation for the 21st Century: Acceptance Limits for Active Pharmaceutical Ingredients (APIs): Part I.
26. Bismuth G, Neumann S,(2000)."Cleaning Validation: A practical approach". Informa Health Care. 200 p.
27. Kalelkar S. "Why the Swab Matters in Cleaning Validation" from http://www.cemag.us/article/why-swab-matters-cleaning-validation?page=0,1
28. Paul Pluta: A Technical Book "Cleaning and Cleaning Validation, Volume 1" from PDA/DHI Technical Book(www.store.pda.org).
29. VidyaSagar G: A Text Book of "Instrumental Method of Drug Analysis" first edition reprint 2010, page no. 479.
30. Potdar MA, A Text Book of "Pharmaceutical Facilities: Design, Layouts and Validation" page no.70-103.

31. Frederick JC, James PA: A Text Book of "Validation of pharmaceutical process" second edition.
32. Khaleeq M Fakhir : "Cleaning Validation Protocol For Pharmaceutical" Pharmaceutical Guidelines (www.pharmaguidelines.com)
33. Justification of limit for cleaning validation in the manufacture of active pharmaceutical ingredient, GMP news. 2007.

For Correspondence:
Waghmare Pradeepkumar V.
Email: pradeep.waghmare90@gmail.com