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Step by Step How to Do Cleaning Validation

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Abstract

It is essential to prevent and reduce the level of cross-contamination in the pharmaceutical industry. Different types of residues need to be considered, including APIs (Active Pharmaceutical Ingredients) residues, degradation products (due to different solubility, toxicity, and cleanability characteristics in comparison with the original compound), particulates, endotoxin, environmental dust, residual rinse water (if product must be dry) as well as potential microbial contaminants[1, 2]. In order to reach this goal, cleaning validation study should be carried out to provide a document which proves that process of cleaning has been validated and it can be performed reliably and repeatedly [3]. In this article we discuss several aspects of cleaning validation, such as bracketing, calculation of the acceptance criteria, swab sampling, rinse sampling, documentation. Additionally, some basic requirements to provide necessities for environmental and equipment cleanliness, before commencement of cleaning validation study, are taken into account. It is worth mentioning that a practical approach is adopted to write this article.

Key-Words: Cleaning validation, Acceptance Criteria, Residue, Swab Sampling, Rinse Sampling

Introduction

Cleaning Validation Definition: Manufacturing processes have to be designed and carried out in a way that prevent cross-contamination as much as possible. Since most pieces of equipment are being used to manufacture different products, cleaning procedure must be able to remove residues from equipment to an acceptable level [4].

Importance and purpose of cleaning validation:

- Not only it is required to comply with regulations, but also it is necessary to fulfill customers' requirements.
- It ensures the safety, identity, strength, and purity of the product which are the basic requirements of cGMP (Current Good Manufacturing Practice).
- It provides manufacturer with enough confidence that internal control is established properly [5, 6]

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It is advisable to perform at least three consecutive and successful applications of the cleaning procedure in order to prove that the method is validated [4].

In case of detecting variable residue, following cleaning (especially an acceptable cleaning), enough attention must be given to effectiveness of the process and operators performance [7].

Equipment cleaning validation maybe performed concurrently with actual production steps during process development and clinical manufacturing. Validation programs should be continued through full scale commercial production [1, 7]. For new chemical entities it is essential to perform a risk assessment analysis before any operation in GMP plants [8].

When cleaning validation is necessary according to guidelines of WHO:

- Product-contact surfaces (Consideration should be given to non-contact parts into which product may migrate for example, seals, flanges, mixing shaft, fans of ovens, heating elements etc.)

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- Cleaning after product changeover (when one pharmaceutical formulation is being changed to another, completely different formulation)
- Between batches in campaigns (when the same formula is being manufactured over a period of time, and on different days). It seems acceptable that a campaign can last a working week, but anything longer becomes difficult to control and define [9].

Cleaning validation for biological drugs must comply with stricter requirements due to their inherent characteristics (proteins are sticky by nature), parenteral product purity requirements, the complexity of equipment and broad spectrum of materials which need to be cleaned [1].

Prevention of cross contamination in production:

- Production in segregated areas or using "closed system" of production
- Providing appropriate air-lock and air treatment system to prevent recirculation or re-entry of untreated or insufficiently treated air
- Providing comprehensive instructions to discharge clothing used in areas where products with special risk of cross-contamination are processed
- Using known effective cleaning and decontamination procedures
- Testing for residues and use of cleaning status labels on equipment [10]

Different types of cleaning:

Different mechanisms are employed to remove residues from equipment such as mechanical action, dissolution, detergency, saponification, and chemical reaction.

Mechanical action: In this method residues and contaminants are removed through physical actions such as brushing, scrubbing and using pressurized water.

Dissolution: It involves using an appropriate solvent to dissolve residues. Water is usually selected owning to being non-toxic, economical, environment friendly and does not leave any residue. However, some residues are only removed by alkaline or acidic solvents. In this case, usage of these cleaning agents is inevitable. Detergency: Detergent acts in four ways as wetting agent, solubilizer, emulsifier, and dispersant in removing the residues and contaminants from equipment. Wetting agents (such as surfactants) decrease the surface tension of cleaning solution, thus they can easily penetrate into the residue. Saponification: This method is based on the breakage of ester bond in fat residue to form fatty acid and glycerol which are soluble in water. For this purpose, some alkalis can be used such as NAOH, KOH. Chemical reaction: Oxidation and hydrolysis reaction chemically breaks the organic residues [6, 11].

Cleaning agents:

Detergents are not part of the manufacturing process. They should be utilized as less as possible and even when they are absolutely required to facilitate cleaning, acceptance limits for cleaning agents residues should be defined. The effectiveness of cleaning procedures for removal of detergent residues should be evaluated. Ideally, no (or for ultra-sensitive analytical test methods-very low) amount of residue should be detected. The composition of detergents should be known to manufacturer and they should ensure that they are notified by supplier of any critical changes in the formulation of the detergent. Detergents should be acceptable to the QA (Quality Assurance)/ QC (Quality Control) departments and no superfluous components such as fragrances and dyes should be included in them. Since most products have ingredients with different solubility characteristics, a suitable combination of cleaning agents would be more effective.

If a detergent or soap is used for cleaning, consider and determine the difficulty that may arise at the time of testing for residues. Separate validation of removal of cleaning agents is not required if the removal of the cleaning agent is included in the validation of the equipment cleaning from process compounds. It is also not required for equipment producing only early intermediates or other residues of chemically synthesized APIs [1, 4, 9, 12, 13, 14].

Cleaning agent parameters to be evaluated:

i. Easily removable (some detergents leave persistent residues such as cationic detergents, which adhere very strongly to glass and are difficult to remove).
ii. The possibility of detergent breakdown should be considered when a cleaning procedure is being validated. Additionally, strong acids and alkalis used during the cleaning process may result in products breakdown which requires to be deemed during cleaning validation.
iii. Materials normally used in the process are preferable
iv. The design and construction of equipment and surface materials to be cleaned
v. Ease of detection

vi. Solubility properties of the worst case product (not only the API, possibly exist in small quantity, but also all the substances present in the formulation)
vii. Environmental consideration
viii. Health and safety consideration
ix. Knowledge gained through experience
x. Manufacturer's recommendation
xi. The minimum temperature and volume of cleaning agent and rinse solution
xii. Availability, etc. [1, 5, 15, 16, 17]

Acceptable amount of cleaning agents: The limit for detergents and cleaning agents, following cleaning, is calculated based on LD_{50} value or 10 ppm criteria, whichever is the lowest. LD_{50} can represent toxicological properties of cleaning agents, but cleaning agents generally accepted in pharmaceuticals feature relatively high LD_{50} which leads to calculation of high acceptable quantities of residues. Therefore, it is reasonable to select the lowest amount between LD_{50} and ppm criteria. Another assessment can be carried out in this way that the amount of the residue does not exceed the detection limit of the method of analysis for the relevant detergent substance. Calculation of cleaning agents residues based on 10 ppm criterion is the same as the calculation of APIs residue based on this criterion.

Limit calculation for cleaning agent residues:

Calculation of the maximum acceptable residue:

$$ADI = \frac{\left(5\times10^{-4}\times LD_{50}\left[{mg}/{kg}\right]\times 70kg\right)}{SF}$$

$$MACO\,(mg) = ADI\,(mg)\times\left({B\,[g]}/{D\,[g]}\right)$$

ADI = Acceptable Daily Intake
SF= Safety Factor which is applied to consider rout of administration
B= Batch Size of the subsequent product
D= Daily dose of the subsequent product
5×10^{-4} times LD_{50} has no measurable pharmacological effects on humans.
70 kg = Average body weight of an adult [18]

Personnel:

Because a manual procedure is an inherently variable method, operators carrying out this method should be properly trained, monitored, and periodically assessed. All training carried out should be recorded [1]. Suitable working clothing is also important to prevent spreading the particles and dust. Since some potentially harmful organisms can be transferred by personnel and products, any direct contact between personnel and products must be avoided. Whether it is inevitable, gloves should be worn [19].

Design and construction:

Buildings and facilities should be located, designed, and constructed to facilitate cleaning maintenance, depending on the type and stage of manufacture. Facilities should also be designed to minimize potential contamination such as microbiological contaminants [10, 13]. In order to reach this goal, all parts of the premises should be made of washable and impervious materials to prevent contaminants from accumulating in cracks and open joints [19].

Equipment parameters to be evaluated:

i. Identification of the equipment to be cleaned
ii. Their design and difficult to clean areas (particularly in large systems that employ semi-automatic or fully automatic system)
iii. Property of materials
iv. Ease of disassembly
v. Fixed or not etc. [4, 5, 20]

Note: All equipment should be designed in a way to permit visual inspection and whenever possible, equipment should be made of smooth surfaces of non-reactive materials, as it may alter the safety, identity, strength, quality, or purity of the drug products [1, 21]. Drain open channels should be avoided and if it is unavoidable, they should be shallow to facilitate cleaning [10].

Dedicated areas: Dedicated production areas include facilities, air handling equipment and/or process equipment. Dedicated equipment should be used for products which are difficult to remove (e.g. tarry or gummy residues in the bulk manufacturing), for equipment which is difficult to clean, for products with a high pharmacological activity or toxicity (e.g. biological or products of high potency which may be difficult to detect below an acceptable limit, certain steroids or cytotoxic anti-cancer agents), or for production of highly sensitized materials, such as penicillins or cephalosporins [4, 7, 20]. Full cleaning validation is not required for products of dedicated equipment. Visual examination and microbial tests are only acceptable if cleaning/ sanitizing agents are not utilized. Location of air discharge and air intake of separate units should not be situated near each other in this area. Personnel are only allowed to remove their protective clothing in the defined areas and then their clothing should be thrown away by wrapping up in a suitable bag [22, 23]. Stringent regulations should be established to prevent

cross-contamination from personnel, materials, etc. moving from one dedicated area to another.

In a bulk process, particularly for every potent chemical such as some steroids, the issue of by-products needs to be considered if equipment is not dedicated [1, 24].

For equipment producing multiple compound types (e.g. final APIs and early intermediates), the most conservative limit for all compound types produced in equipment must be selected [25].

Level / degree of cleaning:

The level or degree of cleaning and validation required for the manufacturing process of drug substances mainly depends on:

i. Usage of equipment (dedicated equipment or not)
ii. Manufacturing stages (early, intermediate or final step)
iii. The nature of the potential contaminants (solubility, toxicity, etc.)

Note: In early production it may be unnecessary to validate equipment cleaning procedures, where residues are removed by subsequent purification steps [26].

Cleaning validation methods for equipment:

i. Manual
ii. Semi-automated
iii. Fully automated

Note: In all methods, cleaning period and number of cleaning cycles must be evaluated and cleaning procedure must be proved to be effective, consistent and reproducible.

Note: Manual methods should be reassessed at more frequent intervals than clean-in-place (CIP) systems [4]. FDA recommends CIP should be used to clean process equipment and storage vessels in order to reproduce exactly the same procedure each time, though the critical points of the CIP systems should be brought under control by using appropriate sensors and alarm systems [1]. It is also recommended when pieces of equipment cannot be separated from each other.

Manual method would be more variable due to its dependence on operators' skills. However in some instances, it may be more practical to use only manual procedures [5].

Equipment Hold-Time:

Clean Hold Time is generally considered to be the time between the completion of cleaning and the initiation of the subsequent manufacturing operation. The time between the end of manufacturing and the beginning of the cleaning process is termed dirty hold-time. It also begins when the clean equipment is initially solid. Dirty equipment is harder to clean, because the dirt on equipment has a greater chance of becoming sticky as hold time increases, especially topical products, suspensions and bulk drug. The length of time between the end of processing and each cleaning step must be evaluated. Sufficient studies should be performed to indicate that all pieces of equipment are cleaned and kept in an appropriate condition to prevent any probable microbial proliferation.

Clean Hold- Time should be established to ensure that clean equipment will stay clean, provided they are stored in an appropriate condition [15, 16, 27]. The dirty- hold time is limited to 7 days and the clean-hold time to several weeks. A less aggressive approach uses the longest hold-time data. This suggests a maximum dirty hold-time of 9 days and a clean-hold time of more than two years. It is necessary to perform a risk assessment study if the dirty validated hold time exceeds and even it may also be necessary to evaluate the product or microbial contamination of equipment following cleaning. If the clean hold-time exceeds, equipment should be cleaned again prior to use and verified as clean[23, 27].

Elements of cleaning validation:

Visually clean: All pieces of equipment which are in contact with products are individually examined (wherever possible) for cleanliness. This visual inspection makes it possible to take samples from areas which are inaccessible. It may contribute to early localization and identification of any improper cleaning procedure [12]. The visual cleanliness of equipment must be checked and verified after cleaning according to the procedure [20, 28]. It is compulsory for every changeover. It also should be included in the validation protocol [22].

Spiking test:

This test is used to valid the visible concentration of residue of the worst case on the surface test. For this purpose, dilute series of the worst case are made in a volatile solvent .The volume should be applied on a select test surface which is similar to the sample surface (e.g. 25 cm^2). To ensure that API quantities are distributed uniformly on the test surfaces, the test must be performed with different concentrations under the same circumstances as much as possible and the approximate volume should be utilized. The visual limit of detection is determined after the complete evaporation of solvents by comparing the test surfaces. However, the most important problem of this procedure arises in this stage. The determination of the visual LOD is strongly influenced by the following

factors: surface characteristics, light intensity, person who carries out the test (acuteness of the vision, subjective rating- visible/ invisible), test execution (eye distance, angle, type and duration of observation), even API distribution

In order to determine the visual limit of detection accurately, the test conditions should be similar to the cleaning validation study conditions as much as possible. For instance, testing on different surfaces like the production equipment to be investigated (different surface roughness, different shapes, different lighting conditions), testing with different personnel. The same standard cleaning procedure should be applied to both equipment and surfaces being examined to determine the visual limit of detection. However, the role of APIs particle distribution can significantly reduce effectiveness of the procedure. In case of particles accumulation they become visible, though the same amount of APIs may not be visible when they are homogenously distributed.

If the maximum allowable carryover is higher than the limit of visually clean test, it is necessary to determine visual limit of detection. However, it is controversial not to perform other stages of cleaning validation apart from spiking studies. In order to achieve a conclusive evidence to use the visual criteria, it is also recommended to take some samples by swabbing or rinsing procedure from visually clean surfaces and determine the actual available residue analytically [18].

If residue is detected during the visual inspection, it should be considered as a deviation which needs to be assessed immediately [29].

For equipment in which Generally Recognized as Safe (GRAS) compounds are manufactured, there is no need to perform visual quantitation [30].

Bracketing or grouping: It is not necessary to valid cleaning procedures individually for all products and processes which are very similar. It is considered acceptable to select a representative range of similar products and processes concerned in production which is termed "worst case" [4, 7]. The worst case should represent the worst condition. This practice is termed "Bracketing "or "Grouping". It includes grouping by product or grouping by equipment. The grouping by product maybe allowed when the similar products are manufactured in the same equipment. Identical cleaning processes should then be used for these products (cleaning agent, cleaning method, process parameters).The worst case product is the most difficult product to be cleaned [4, 9]. In case of determining two products as worst cases within a group, there are two conditions: a) If these two products are equivalent, a lot combination of these products are used to satisfy the three validation cleanup requirement (e.g. three of product A or B, two of product A and one of product B or one of product A and two of product B). b) If they are not equivalent, three validation cleanup procedures are required for each worst case [31].

Grouping by equipment maybe allowed if it is similar equipment, or the same equipment in different sizes. An alternative is validating separately by using the smallest and the largest size [9].

A worst case determination study should be based on:

- Active product solubility, toxicity, and potency (less soluble; more potent; and more toxic product, more worst the situation)
- Active product detectability
- Lowest batch size, more worst the situation
- The maximum daily dose of the next product (highest daily dose, more worst the situation)
- The number of dosages that can be made from next batch
- Lowest strength of previous product, more worst the situation
- The total area with which product comes into contact (largest contact surface area, more worst the situation)
- The area of one tablet or the volume of one individual fill, and
- The API content in the product [12]

It would be a helpful suggestion to perform a risk analysis study and categorize the products to select the worst case.

First, we should categorize the products based upon their toxicity. Then, we should categorize the products based upon their solubility. Finally, each product will be incorporated into one part of the table 5 [32].

Note:

i. Effectiveness of cleaning procedure to remove all residues is not restricted by cleaning validation study, i.e. low toxicity should not be a reason for improper cleaning [10].
ii. If there is an insoluble pigment, such as film coating, validation of cleaning procedure should be done but it is restricted to the coater. The visually clean method must be performed here [18].

Establishment of the acceptance criteria:

In order to select rational limits for products residues, scientific consideration should be given to the materials, the facilities, the contaminant type (solubility of the potential residue, difficulty of cleaning, stability, their therapeutic dose, application of

the product, in addition to the risk to operators, products and patients), nature and batch size of all the products manufactured in the same equipment. The limits should be practical, achievable and verifiable. Sensible limits can only be imposed by considering the sensitivity of the analytical methods.

The approach for setting limits can be: Product specific cleaning validation for all products, grouping into product families and choosing a "worst case" product, grouping into groups of risk (e.g. very soluble products, similar potency, highly toxic products, difficult to detect), setting limits on not allowing more than a certain fraction of carryover, different safety factors for different dosage forms.

Carry-over of product residues should meet defined criteria, for example the most stringent of the following three criteria:

i. No more than 0.1% of the normal therapeutic dose of any product will appear in the maximum daily dose of the following product (according to the ICH impurity document which indicates that up to 0.1% of an unknown individual or 0.5% of total unknown material may be present in the product being tested).
ii. No more than 10 ppm of any product will appear in another product
iii. No quantity of residue should be visible on equipment after cleaning procedures are performed. Spiking studies should determine the concentration at which most active ingredients are visible.

It is not feasible to ensure that the contaminant is uniformly distributed in whole system or will be uniformly removed from the system. It would also be a wrong presumption that the contamination only occur at the beginning of the batch [4, 7, 13, 15, 20, 26].

If the acceptance limit is lower than the analytical limit of detection, equipment must either be dedicated, or an alternative, more sensitive method of detection must be developed. So for certain highly sensitized or highly potent ingredients the limit should be below the limit of detection by the best available analytical methods, dedicated plants used for these products are more practical [5, 22].

For Non-Therapeutic materials the Residue Acceptance Limit is based on toxicity [23].

For particular situation justifiable acceptance criteria should be determined according to each company's requirement [33].

Calculation of the Maximum Allowable Carry-Over (MACO):

Dose criterion:

$$MACO\,(mg) = SF \times LHDp\,(mg) \times \frac{BSs\,(g)}{IFs \times MDs\,(g)}$$

SF= Safety Factor

LHD_P = Lowest Human Therapeutic Dose of the Previous product

BS_s = Batch Size of the Subsequent product

IF_s = Intake Frequency of the Subsequent product

MD_s = Mass of the Dosage form of the Subsequent product

Advantage of this criterion:

The pharmacological properties of the API are considered in the calculation by applying therapeutic dose of the drug substance.

Disadvantages:

i. Due to consideration of different subsequent products for the worst case product, it is expensive and complicated. Thus it is reasonable to calculate the limit for 2 real "worst case" conditions, the product with the largest daily dose and the product with the smallest batch size. The smaller of these two limits is used for the cleaning validation.
ii. The calculated limits are proportional to the therapeutic dose. So APIs with a low therapeutic dose result in low limits, and APIs with a high therapeutic dose result in high limits. It is probable that sometimes these high limits exceed the visual limit of detection and contravene the visually clean criteria which contradicts the general GMP requirements [18, 26].

For considering 10 ppm as the acceptance criteria:

The quantity equivalent to 10 mg/L of the batch size is considered as the acceptance criteria for the acceptance criteria as 10 ppm. It is a pharmacopeia limit test which is useful for materials for which there is no available toxicological data. It is also used for calculating the acceptance criteria for heavy metals in starting materials [16, 17].

$$MACO\,(mg) = 10ppm\left({}^{mg}\!/_{kg}\right) \times BSs\,(kg)$$

BS_s = Batch Size of the Subsequent product

Advantages of this criterion: Since there is no need to calculate the limit for each previous product/ subsequent product combination, calculation is easier. The worst condition can be considered if the smallest

possible batch size of the subsequent product is used for calculation.

Disadvantages: Since the therapeutic dose of the API is not included in the limit calculation, the various pharmacological properties of the different APIs are not taken into account. Thus for highly potent drug substances this criterion is not acceptable from a pharmacological point of view [18].

Calculation of the acceptance criteria for swab samples:

Active Ingredient Residue (for Non-dedicated equipment): Acceptance criteria based on the following rationale for swab samples:

$$Limit\left(ppm\right)=MACO\times\frac{1000}{C}\times\frac{D}{V}$$

Where,

C-Cumulative surface area of equipment used (in cm^2).

V-Volume of solvent used to dispense swab.

1000-Multiplication factor to convert value in mcg from mg.

D-Swabbed Surface Area in cm^2

Calculation of acceptance criteria for rinse samples:

Active Ingredient Residue (For Non-dedicated equipment): Acceptance criteria based on the following rationale for rinse samples:

$$Limit\left(ppm\right)=MACO\times\frac{1000}{C}\times\frac{1}{V}$$

Where,

C-Cumulative surface area of equipment used (in cm^2)

V-Volume of solvent used for rinse of the same surface in mL per cm^2 of equipment

1000-Multiplication factor to convert value in mcg from mg [34]

Note:

i. Safety Factor is a measure of degree of risk for a particular situation [26]. It is applied during calculation to ensure that the level of product carryover is low enough not to have a pharmacological effect [22].
ii. If some pieces of equipment are utilized repeatedly, for each usage their surfaces should be involved in the calculation of total product contact surfaces [18].

Sampling:

Sampling should be performed according to the cleaning validation protocol. There are two main methods of sampling, direct surface sampling (swab method) and indirect sampling (use of rinse solution). The selection of either of these techniques must be scientifically justifiable and fulfill the aim of the study which is to demonstrate that the amount of residual material in equipment has been reduced to an acceptable level. A combination of the first two methods is generally the most desirable, particularly in circumstances where some pieces of equipment are not sufficiently accessible to perform direct sampling. FDA prefers swab sampling to rinse sampling.

The selection of an appropriate extraction solution is an important step in establishing a swab or a rinse procedure. In order to select a suitable extraction solution, solubility of the target residue should be assessed in the select solution. Various alcohols, water, buffers, or combination of them are common extraction solutions used for cleaning validation procedures.

Factors which need to be considered to select a suitable sampling method: design of equipment (accessibility), solubility of the residue, suitable and available analytical methods

Swab sampling:

A probe with an inert material (usually cotton, wool or polyester) that is moisturized with highly pure water, such as water for injection (WFI) is used in this method. Then this should be rubbed methodically across a surface (e.g. 25 cm^2).

Since it is not possible to take swab samples from the whole equipment, sampling locations should be selected from the worst places where residues are more likely to exist. Then the result is extrapolated to account for the total contact surface area, though this may result in wrong calculation of the residue because the result may exceed the maximum allowable carryover (since it is more probable that the residue remains in areas which we take sample from). Sampling locations should be labeled by suitable materials which are flexible enough to adapt to the surfaces, and also inert materials which are resistant to solvents (such as foils made from PTFE or silicone). Use of adhesive strips to fix the labels to the surfaces should be avoided, since they leave residues when they peel off. Stencils should be used for a particular API or they must be cleaned after usage [1, 4, 5, 9, 11, 15, 33, 35, 36, 37, 38].

Swab sampling technique:

To ensure that the select sampling techniques are suitable to accomplish the calculated acceptance criteria, prevalidation studies and method development must be performed. A standard swabbing motion is essential to neutralize differences between operators in swabbing procedure which leads to non- replicable recovery. Swabbing patterns can vary and are dependent on the surface or equipment being swabbed. The operator conducting a swabbing procedure must follow a series of steps:

Pretreat the swab(s) in the sample of solvent, and squeeze the swab(s) to remove the excess solvent from the swab head→ Swab the surface firmly and evenly with one side of the swab(s) in a horizontal direction, and with the other side in a vertical direction back and forth, one stroke back and one stroke forward, always going from clean to dirty areas (another variations involves overlapping zigzag strokes in opposite directions, making sure that the swab head never leaves the surface being evaluated. An easy way to do this is first, horizontally and second, vertically). → Cut off the swab head into a suitable vial, then seal and label it → Use 10ml of sample solvent (also called recovery solvent or extractable solvent) to extract the drug residue by sonication→ Filter the extracted sample and analyze the sample by a suitable analytical method → Compare the results with the blank samples produced by moistening the swabs in the same solvent that were not rubbed on the surface being tested Note: Using a dry swab after the wet swab can aid to collect any remaining solution on the surface swabbed. Special surfaces or pieces of equipment may require other swabbing patterns to maximize percentage of recovery.

Some points to be considered include:

The number of swabs used, shape of swabs (the long swabs with small heads are excellent for general purpose and hard to reach area. Other swabs with larger heads are better suited for sampling of broad, flat areas), area swabbed, wet or dry swabs, the amount of solvent on each swab, the exact motion of the swab over the surface, the number of strokes over the sampling site, the amount of time spent at swabbing and sonication, type of the filter and solvent (the residue should have good solubility in the solvent chosen and should not degrades in the solvent), the percentage recovery of the swab extraction procedure, the effectiveness of the swab at recovering residues from equipment parts surface, the suitability of the material and its interference in the analysis (the adhesive used in swabs has been found to interfere with the analysis of samples, hence it is better to pretreat the swabs heads with the solvent used later to prepare the samples).

Filtering efficiency is measured through dividing the amount of residue in the filtered sample by the amount of residue in the unfiltered sample and expressed as a percentage. The pH value plays a role in filtering efficiency. It would be desirable to choose the solvent used in the subsequent analysis (e.g. HPLC) as the extractable solvent whether the filtering efficiency and the percentage of recovery are not influenced negatively.

According to a research study, higher recovery would be achieved by using two swabs to take sample from each coupon. But the volume of solvent soaking on to the swabs tips is very important. This volume varies because of difference between operators and individual stroke.

The use of two swabs increase the efficiency of absorbing drug residue relatively but not dramatically due to more solvent used on the tips. Thus the use of more than two swabs would not probably be a good way to improve recovery due to increase in volume of solvent using on the swab tips. The use of two swabs is an ideal squeezing method. According to one study, there is no significant difference between fingers or against the beaker wall to squeeze swabs in order to extract residues [9, 35, 37, 38, 39, 40].

In swab sampling method a level of contamination or residue per given surface area is established. Where individual swab result(s) is/ are greater than the acceptance criteria, it is then necessary to calculate the MC (Measured Carryover) to demonstrate cleanliness. An individual swab result is allowed to be greater than the permitted amount as long as the MC is less than the MACO. However, it is recommended that no individual swab should be greater than × 10 the permitted amount for each swab (× 10 is safety factor built into the calculation to cover uncertainty in the sampling and determination of carryover).

MC: It is calculated by adding the individual swab results [22].

$$MC = \frac{\text{sum of swab values} \times \text{total surface area of whole equipment}}{\text{total surface area sampled by all swabs}}$$

Rinse sampling:

Two different procedures can be utilized as a suitable method for rinse sampling. a) A test which measures the amount of residue in the solvent used for final rinsing of equipment (thoroughly wet all product contact surfaces and circulated through all product contact lines). b) A test for the additional rinse volume used on clean equipment after the final rinse (solvent rinse).

To perform sampling reproducibly, always the same volume of solvent should be used. The total volume of the final rinse can be calculated by circulating rinsing water in a close system or collecting quantitatively.

Non-standard rinse volume: where it is not possible to rinse to the required ratio of Rinse: Surface area, the actual volume used is recorded and an adjustment to the acceptance limit is made. For example, if the rinse

volume required for sampling is twice as high as the calculated volume, the limit is then halved [37, 41].

As a norm, Rinse sampling should be used in combination with other sampling methods such as swabbing. This is an easier method in comparison with swabbing. It may be necessary to determine:

i. The effectiveness of the rinse solution at recovering residues from equipment parts surfaces
ii. The interference of the rinse solution in the cleaning procedure and analysis
iii. The acceptable residue concentration should be above the LOD. Hence analytical LOD must be determined before the volume used for rinsing equipment and sampling preparation is defined.
iv. Temperature of the wash and rinse water or other solvent(s)
v. Flow rate and/or pressure at which the wash and rinse solvents are delivered
vi. Volume or amount of water or other solvents used to wash and rinse equipment

Water for injection should be used as the last rinse for product-contact equipment to be employed to manufacture sterile products. Purified water is considered acceptable as the last rinse for product-contact equipment used in production of non-sterile products.

Note: Tap water is not considered suitable to be used in the last rinse of any cleaning procedure for product-contact equipment due to variable levels of organic and inorganic residues such as chlorine that may exist in it.

In case of using an organic solvent for rinse method, some issues such as danger of explosion should be considered [1, 15, 33].

How to perform rinse sampling?

For closed systems such as pipes, containers:

Fill equipment with the specified volume of solvent→ set equipment in operation for the specified period of time or allow the solvent to circulate→ take sample

For open systems such as sieving machine:

Rinse equipment parts with specified volume of solvent→ collect the solvent in a container→ take sample

Smaller parts of equipment can be placed in a defined volume of the solvent for a defined period of the time then the sample can be analyzed [37].

Containers for collecting samples must be clean and thoroughly rinsed with distilled water, especially when taking for conductivity test or TOC analysis. Containers used to transfer water samples through product lines must also be clean and rinsed thoroughly with distilled water. For TOC test it is important to collect a small sample of the rinse water used as a blank sample [31].

Other methods for sampling:

Other methods of sampling include steam condensation method, placebo sampling, routine production In-process control, and FTIR.

i. Steam condensation method: Since hot steam can penetrate into all parts of equipment, amount of residue can be measured in the collected steam which condenses. Hot steam is suitable for lipid soluble APIs and cleaning agent residues due to its good solubility characteristics. Nevertheless, it is just usable for closed autoclavable production equipment.
ii. Placebo sampling: It can be used to detect residues on equipment through the process of a placebo batch subsequent to the cleaning process. This method is based on choosing a placebo rather than the main API selected as the worst case. Attributes of the placebo should be similar to the selected product. Batch size of the placebo is affected by the equipment characteristics. Placebo method should be used in conjunction with rinse and /or swab methods [1, 9, 11, 13, 15, 33].
iii. Routine Production In-Process Control: Monitoring-Indirect testing, such as conductivity, may be valuable for routine monitoring once a cleaning process has been validated. These methods can be applied to bulk drug manufacturing where sampling is only viable by using rinse solution such as reactors, centrifuges, and piping between such large equipment. Any indirect test method must have been shown to correlate with the condition of equipment [1, 13].
iv. FTIR or photoelectron emission techniques: These techniques are based on direct measurement of spectra obtained from the residue remaining on the surface. In this method sampling and analysis both occur in 1 step [42].

Recovery studies:

The recovery study is being carried out to evaluate quantitative recovery of the residue from both the surface to be sampled and the sampling method. It is used to define how capable the select sampling method is of recovering the "seeded" drug substance from the clean surfaces, and how capable the analytical method is of identifying the drug substance accurately and reliably in combination with the sampling method.

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Recovery is the percentage of residual material that is actually removed by the sampling technique.

$$\text{percentage of recovery} = \frac{\text{the amount recovered from each surface}}{\text{the amount seeded}} \times 100$$

Then the final results of cleaning validation study should multiply by this percentage to achieve the actual quantity of the residue remaining on the surfaces.

Note: Because of the individual difference in solubility of residues, the solvent used, the nature of the manufacturing surfaces, and the difference between analytical methods, however, it is generally not possible to achieve recovery beyond a certain level and establish acceptable fixed limits. FDA guidelines recommend a minimum of 50% recovery.

For some products percentage of recovery may be as low as 10-20% due to low solubility such as proteins. For soluble residue, a higher percentage recovery would be expected.

Recover study method: A surface equivalent (material, polish grade) to equipment surface to be cleaned should be spiked with a known amount of substance. This should be analyzed by the same sampling and analytical methods which will be used for the cleaning validation study. The time between applying the solution on the coupons and sampling should corresponds to the maximum time defined between the end of operation and commencement of cleaning procedure. The overall measured results of this procedure are then compared to the actual amounts applied to the surface being sampled or comparable surface. This should be performed at, above, and below the acceptance limit in the test solution. It is important that the reference solution is prepared with the same solvent used for sampling. In order to ensure that the recovery test is carried out in a reproducible way, the recovery test should be repeated three times for each amount seeded. Then the average of three recovery percentages is applied to calculation [9, 15, 28, 35, 43].

Analytical methods:

The analytical methods should be validated and documented before the cleaning validation study is carried out unless they are included in the relevant pharmacopoeia or other recognized standard reference [20].

The basic requirements are:

i. The ability to detect the target substance(s) at levels consistent with the acceptance criteria (sensitivity).
ii. The ability to detect the target substance(s) in the presence of other materials that may also be present in the sample (selectivity). For example, materials which interfere with UV spectrum. In the event that such interference in the assay is unavoidable, it must be assessed before commencement of cleaning validation procedure and if necessary, quantitative analysis should be carried out. The potential for interferences are swab extractable, cleaning agents, excipients and other potential compounds, sample containers and lids.
iii. The analytical method and the percentage of recovery of contaminants should be evaluated when they are employed in combination with the sampling methods. This is used to show whether the contaminants are likely to be recovered from the equipment surfaces. This is necessary to draw a logical conclusion based on the sample results, though poor sampling method may also result in a false-negative result.
iv. These parameters should be checked: Precision, accuracy, linearity, range, Limit of detection (LOD), Limit of quantitation (LOQ), recovery by spiking with the analyte, and reproducibility.
v. 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.
vi. Stability of samples overtime as samples integrity may be affected by the time interval between removal and testing of samples.
vii. The method shall be practical and rapid, and, as much as possible use instrumentation existing in the company [4, 5, 15, 33, 44, 45].

Note: An analytical method with an LOD of at least 25% of the target residue limit in the analyzed sample is preferable since it increases the safety of procedure.

Specific and non-specific methods are widely used to detect any compound. The analytical method is selected based on characteristics of the residue and the analytical limits calculated for the residue. Some characteristics of the residue which need to be considered are whether the residue is organic or inorganic, is soluble in water or other solvents, its degree of polarity, and its stability in the cleaning environment. The select method should broadly leads to generation of a logical and scientific result related to the residue. The choice of using a specific or non-specific method can be difficult. If a drug residue is

highly toxic, a specific method is always recommended.

Specific method: It is a method that detects the unique compound in the presence of potential contaminants. Some examples of specific methods are high performance liquid chromatography (HPLC), Ion chromatography, Atomic absorption, Immune assays, Capillary electrophoresis, flame photometry, enzymatic detection and other chromatographic methods.

Non-specific methods: It detects any compound that produces a certain response. Some examples of non-specific methods are Total Organic Carbon (TOC), pH, titration, and conductivity. A non-specific method is deemed acceptable where total contaminants are being studied as opposed to just a specific contaminant. A non-specific method is employed based on this assumption that the select worst case represents the whole amount of residue in the sample, although the real percentage of the worst case residue in the sample analyzed cannot be measured. It should be noted that the specifity of a method is not an absolute property but it depends on possible interferes (9, 11, 39, 45).

Note:

i. It is always sensible to choose the simplest technique that can be used to reach the desired goal.
ii. If levels of contamination or residue 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.
iii. When more than one impurity is suspected (which is probably the normal case in API manufacturing) it is not necessary to employ a specific method for each impurity. Only a method that can detect all impurities together is deemed appropriate. Then it should be presumed that the worst case impurity indicates the whole residue. It would be acceptable by authorities and acceptable for patients' safety. It is also a practicable approach for the industry because such methods (for example dry residue determination for non-volatile impurities or TOC determination for water rinses) are very simple methods.
iv. In the case of biological drugs, the use of product-specific assay(s) such as immunoassay(s) to monitor the presence of biological carry-over may not be adequate. A negative test may be the result of denaturation of protein epitope(s). Product-specific assay(s) coupled with total organic carbon (TOC) can be employed for detection of protein residue.
v. Interferences from the sampling procedure must be taken into account: This should include blank extractions of the swab material. Aged samples can be used to evaluate whether the target substance altered during cleaning procedure. Specifity is usually demonstrated by sufficient chromatographic resolution, or lack of interference (1, 5, 13).
vi. Due to long hydrocarbon chains without functional groups existing in the structure of the organic compounds they cannot be detected by using UV/VIS detectors (43).
vii. Optically Simulated Electron Emission can be employed where limits of the residues are very less that make it impossible to be detected by conventional methods. It can be used for both qualitative and quantitative analysis (42).

Microbial aspects

Sampling for microbial analysis should be performed prior to swab and rinse sampling to prevent false positive results from the proceedings.

Evaluation the potential for microbiological risk:

i. The existence of favorable conditions to reproduction of microorganisms (e.g. moisture, cervices and rough surfaces) should be considered. To provide confidence that routine cleaning and storage of equipment is adequately appropriate not to allow microbial proliferation, the period and when appropriate, conditions of storage of equipment before cleaning and the time between cleaning and equipment reuse, as well as time frames and conditions for the storage of clean equipment should form a part of the validation of cleaning procedure. Equipment should be dried before storage and under no circumstances should stagnant water be allowed to remain in equipment subsequent to cleaning operations (4, 13).
ii. Integrity of the vessel prior to manufacture and nature of materials manufactured in equipment should be assessed (28).
iii. Use of organic solvent or other conditions which prevent microbial growth and survival (e.g. high temperature, high or low pH, etc.) (47)

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Note:

i. Prevention of microbial contamination is preferable to removal of contamination once it has occurred.
ii. Risk of endotoxins, resulting from killed gram negative bacteria, which may transfer to parenteral dosage forms should be evaluated.
iii. Whether or not CIP systems are used for cleaning of processing equipment, microbiological aspects of equipment cleaning should be considered [4, 47].

Microbiological samples should be collected prior to and throughout the cleaning procedure to assist in selection and confirmation of the efficacy of disinfectants and detergents. Since raw materials, intermediates as well final products are deemed as possible sources for microbiological contamination, all pieces of equipment that come in contact directly with these materials must be evaluated from a microbiological point of view. Sampling frequency greatly depends on historical data, types of dosage forms manufactured, and susceptibility of the products. The cleaning validation techniques for microbial evaluation include swab method, surface rinse method, contact plate method [5, 15, 21]. Swabs are made of sterile cotton, wool or calcium alginate. Swabs are useful for checking the cleanliness of curved pieces of equipment, pipes and valves.

Collecting Microbiological samples by:

i. Swabs: The sample containers are pre-sterilized→ the sample valves used are cleaned and pre-sanitized by flushing with 80° C distilled water for 5 minutes→ Sterile cotton swabs will be moistened with sterile peptone water, WFI, or phosphate buffer→ Then the swabs are rubbed against the surface (generally, diameter range is 24-30 cm^2) to take sample of a predetermined area→ The swab is then aseptically transferred to a sterile container containing a suitable diluent → The samples are agitated to release all microorganisms into solution (a solution containing 1% sodium hexametaphosphate can be applied to release the organisms from swabs heads)→ The serial dilutions are plated on growth media for quantitation → The plates are incubated for 2 days at 30°-35°c followed by an additional incubation of 3-5 days at 20°-25°c→ Microbial counts are reported per swab .
ii. Contact plates and film: Agar plates (for flat surfaces) or film (for curved surfaces) which were brought out to room temperature and kept in a closed state, should be pressed on to the area to be sampled for approximately 5-10 seconds and immediately sealed→ the samples are incubated such as the swab method→ finally, the sampled surface must be disinfected by using 10% alcohol to prevent any probable microbial growth due to agar residue. This method is suitable for checking flat surfaces and also results in accurate assessment of in situ microbial status.
iii. Surface rinses: surfaces are washed with buffer or media→ Dilutions are provided and plated on different media→ Incubation is performed such as the above methods

This method is suitable for irregular surfaces, particularly when the other two methods are difficult to use [19, 21, 23, 37, 48].

Note: The time when the sampling is carried out is decisive when determining the number of organisms.

It is important to determine the type of present organism. . Bacterial and fungal species selected should be representative of environmental, human, and sources of material microbial flora. Microbial species, which are resistant to antimicrobial agents, should also be considered. It is necessary to demonstrate the absence of pollution indicator organisms such as, Escherichia coli, Salmonella SPP, and Pseudomonas aeroginosa, from all locations monitored. Although it should be noticed that high levels of other microbial flora do not mask these organisms [15, 49].

Documentation:

Cleaning procedure instruction:

The cleaning process should be documented in an SOP (standard operating procedure), including a complete description of the methods and materials, manufacturing system or each piece of equipment and if necessary, the methods of disassembling and reassembling as it is necessary to ensure proper cleaning, all routine monitoring, number of cleaning cycles to be performed consecutively, choice of cleaning agent, the person who carried out the cleaning, cleaning and maintenance schedules, when the cleaning was carried out, the product which was previously processed on equipment being cleaned and instructions for removal or obliteration of previous batch , instructions for protection of clean equipment from contamination prior to use, inspection of equipment for cleanliness, immediately before use, if practical; and establishing the maximum time that may elapse between the completion of processing and equipment cleaning when appropriate [4, 15, 20, 21, 50, 51].

The cleaning record should be signed by the operator who performed the cleaning and by the person who is responsible for the production. It should be approved by the Quality Assurance unit [4].

If any deviation from the planned cleaning procedure occurs on one of the replicate runs, the reason and source of this deviation should be determined immediately. This stage can be removed from the sequence, providing an assignable cause can be determined and the deviation is unrelated to the cleaning procedure, e.g. power loss, cleaning utility malfunction. Otherwise it would be necessary to evaluate cleaning procedure. Then it should be followed by three successful cleanings [23].

Cleaning verification:

Study of monitoring the cleaning activity before completion of the three cleaning cycles on commercial batches of the product shall be considered as cleaning verification [34].

Cleaning validation protocol:

A cleaning validation protocol is required to lay down that how the cleaning process will be validated. It should be written before the practical work for cleaning validation commences. It should include or reference the documents required to provide the following information:

The objective of the validation process

- Scope of the study: The firm should decide that for which residues the cleaning validation study must be carried out based on sound scientific rationale, how many times the study should be run before a report is completed. Sterilization is not included, except that reduction of the biological material will lead to successful sterilization and minimization of pyrogens.
- Responsibilities for performing and approving the validation study.
- Precleaning
- Description of equipment to be used (complexity, design of equipment, disassembly and size of the system, model, serial number or other unique code) and products to be removed
- Staff training
- The interval between the end of production and the beginning of the cleaning procedure
- Cleaning procedures to be used for each product, each manufacturing system or each piece of equipment
- The number of cleaning cycles to be performed consecutively, cleaning agents and its concentration , soakage time, solution volume, water quality, time and temperature, flow rate, pressure, and rinsing
- Any routine monitoring requirement and list of equipment used for this purpose, listing of the process parameters to be verified (this is particularly necessary when automated or semi-automated cleaning techniques are to be employed).
- Clearly defined sampling locations, sampling procedures, including the rationale for why a certain sampling method is used, how many samples are to be taken and any particular requirements should also be stated i.e. for sterile sampling/sampling light sensitive products
- Data on recovery studies where appropriate
- The acceptance criteria, including the rationale for setting the specific limits
- Analytical methods including the limit of detection and the limit of quantitation of those methods
- Other products process, and equipment for which the planned validation is valid according to a "bracketing" concept
- When revalidation or change control will be required
- The protocol should indicate that a summary report is to be written once the validation procedures are completed [2, 4, 16, 17, 31, 33].

The cleaning validation protocol should be formally approved by the manager to ensure that aspects relating to the work defined in the protocol are known and acceptable. Quality Assurance should be involved in the approval of the protocols and reports [4].

Cleaning validation report

A validation report is necessary to present the results and conclusions and secure approval of the study. The data should indicate that residues have been reduced to an "acceptable level". The report should include the following:

Cleaning procedure must be approved by responsible staff. Any deviation from the protocol should be assessed.

In cases, where it is not probable that further batches of the product will be manufactured for a period of time, it is advisable to provide interim reports on a batch by batch basis as the cleaning validation study has been completed (typically, in Active 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) [4, 5, 6, 11, 51].

Change control/ revalidation

A change control system is required to provide confidence that all changes that might impact the cleaning process are assessed and documented. Minor changes or changes having no direct impact on final or in-process product quality should be dealt with through the documentation system. When implementing approved changes, all documents affected by the changes should be revised [1, 4, 11, 20].

Revalidation: Revalidation is necessary to ensure that influence of any proposed changes on the valid cleaning procedure are completely taken into account. The proposed revised procedure may need to be evaluated prior to routine implementation. When there is no intentional change to a procedure, it is reasonable to assume that a properly trained operator or, a properly qualified automated system will be able to execute the procedure reproducibly and obtain the desired outcome. There are 2 basic categories of revalidation: a) revalidation in case of a known change (changes which influence product quality), b) periodic revalidation carried out at scheduled intervals [1, 9, 11].

Periodic revalidation: It is required as process changes may occur gradually over a period of time or because of wear of equipment. Nature and significance of the changes determine the extent of revalidation. The required minimum to perform revalidation of cleaning procedure is three cleaning cycles. It should at minimum include change control documents and deviation reports, although cleaning effectiveness after each cleaning episode must be verified visually. In addition, effectiveness of automated cleaning systems must be evaluated. Whether no significant changes are observed, all necessary evidence to prove that the procedure remains valid should be provided. Results must be recorded in batch records.

The following points should be considered when periodic revalidation is performed:

i. Review the master formula and specifications
ii. Check the calibration records
iii. Review the SOP
iv. Review the cleaning records
v. Review the analytical methods
vi. Review the records regarding planned preventive maintenance

If any of the following changes, the process becomes invalid, even if the finished product meets the marketing authorization specification for finished products:

i. Changes of starting materials (physical properties, such as density, viscosity or particle size distribution or impurity profiles may affect the process or product)
ii. Change of starting material manufacturer
iii. Changes of packaging material
iv. Changes in the formulation and/or process of products (e.g. mixing times, drying temperature)
v. Changes in equipment (e.g. addition of automatic detection systems, installation of new equipment, major revisions to machinery apparatus and breakdowns)
vi. Production area and support system changes (e.g. rearrangement of areas, new water treatment method), changes in the sequence of cleaning cycles or maximum time interval between use and cleaning
vii. Appearance of negative quality trends, and appearance of new findings based on current knowledge, e.g. failure during cleaning verification/ validation
viii. New products
ix. Changes in the formulation of detergents or new detergents
x. Changes in the type of swab or swabbing pattern
xi. Changes in the analytical procedure
xii. Number of batches in a campaign [1, 9, 23, 29, 48]

Deviation:

Enough attention should be paid to any deviation from the protocol. The following is the way of managing any deviation which may arise during or after cleaning validation: a) Description of the deviation b) Evaluation of the effect of the deviation on product quality c) Definition of corrective actions [29]

Conclusion

To perform cleaning validation study efficiently, all relevant departments such as QA, QC, and production departments should be involved. This study should be carried out according to a perfect schedule. After determination of residue type, appropriate methods must be employed to collect samples of the residue. Then these samples will be analyzed by a suitable method and results will be compared with the acceptance limits calculated before commencement of the study. It is also essential to take the necessity of revalidation into account as applicable.

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Table 1: levels of cleaning [26]

levels		Validation required
Level 2	Changeover of one API to another API Changeover of any intermediate to any API Changeover from early steps to final step of the same product Product changeover of equipment used in final step	Yes-essential
Level 1	Changeover between intermediates of one product to final/ intermediate of another Change in early step to intermediates of another product Changeover from early steps to final step of the same product	Progression between level 0 and 2 depends on process and nature of contaminant based on scientific rational. General limit as the acceptance criteria= 500 ppm
Level 0	Batch to batch changeover in an identical process (the same intermediate and API) Change to early steps of another product	No validation required, although cleaning intervals and methods should be determined. It is necessary to determine maximum campaign length after which cleaning must be carried out

Table 2: Form of test solutions for spiking studies [18]

Solution number	1	2	3	4
Concentration				
Test area				
Volume applied				
Applied quantity of API				

Table 3: Classification of products according to their toxicity [32]

LD_{50} (rat or mouse)	Category
< 200 mg/kg	High
200-2000 mg/kg	Moderate
> 2000 mg/kg	Low

Table 4: Classification of products according to their solubility [32]

Descriptive term for solubility according to USP	Category
Very Soluble, Freely Soluble, Soluble	High Solubility (<30 ml/g)
Sparingly Soluble, Slightly Soluble	Moderate Solubility (30 – 1000 ml/g)
Very Slightly Soluble, Practically Insoluble or Insoluble	Low Solubility (> 1000 ml/g)

Table 5: Worst case determination according to solubility and toxicity [32]

Risk factors		Solubility		
		Low	**Moderate**	**High**
Toxicity	**High**	High	High	Moderate
	Moderate	High	Moderate	Moderate
	Low	Moderate	Moderate	Low

Table 6: Safety Factors [26]

Dosage Form	Safety Factor
Parenteral products	1000-10000
Oral dosage forms (tablets, capsules etc.)	100-1000
Topical products	10-100

Table 7: Advantages and disadvantages of swab sampling [1, 5, 9, 11, 33]

Advantages	Disadvantages
Dissolve and physically remove sample Adaptable to a wide variety of surfaces Economical and widely available May allow sampling of a defined area Applicable to active, microbial, and clean agent residues Residues that are dried out or are insoluble can be sampled by physical removal	An invasive technique that may introduce fibers Results may be technique dependent Swab material and design may inhibit recovery and specifity of the method Evaluation of large, complex and hard to reach areas is difficult(e.g. crevices, pipes, valves, large vessels)

Table 8: Advantages and Disadvantages of Rinse Sampling [4, 5, 9, 11, 13, 16, 17, 37]

Advantages	Disadvantages
Adaptable to online monitoring ▪ **Easy to sample** ▪ **Non- intrusive** **Less technique dependent than swabs** **Applicable for actives, cleaning agents and excipients** **Allows sampling of a large surface area** **Allows sampling of a unique (e.g. porous) surfaces and inaccessible areas of equipment that cannot be routinely disassembled**	Limited information about actual surface cleanliness in some cases Very low amount of residues may not be homogeneously distributed Rinse volume is critical to ensure accurate interpretation of results, since the sensitivity of the assay may be greatly reduced by dilution of the contaminant Sampling methodology must be defined since rinse sampling method can influence results May be difficult to accurately detect location of residues and control the areas sampled, therefore, usually used for rinsing an entire piece of equipment, such as vessel Reduced physical sampling of the surface especially for dried out residues Solubility of the contaminant in the relevant solvent should be considered which may result in lower amount

	of residue in the sample in comparison with real amount on the surfaces

Table 9: Advantages and Disadvantages of placebo method (9, 11)

Advantages	Disadvantages
Placebo contacts the same surfaces as the product Applicable for hard-to-reach surfaces Requires no additional sampling steps It is appropriate for active residues, cleaning agent, particulates and microbial testing It is useful when the worst case is either toxic or hazardous	Lowers analytical specifity and detectability due to dilution of the contaminant Time- consuming and expensive method since equipment must be cleaned after the placebo run Placebo must be appropriate for each potential product Residues may not be homogenously distributed in the placebo, particularly if the contaminant or residue is of large enough particle size. It may lead to wrong determination of recovery No direct measurement of residues on product contact surfaces It is difficult to ensure that the placebo would be removed from the equipment surface uniformly

Table 10: Acceptance Recovery According to WHO Guidelines

%Recovery	Evaluated as
> 80	Good
>50	Reasonable
<50	Questionable

Table 11: Different methods of analysis for cleaning validation (9, 38, 39, 42, 45, 46)

Method	Advantages	Disadvantages	Additional information	Applications
HPLC	Separation of multiple compounds, Several extraction solution can be used, Producing specific peaks of interest , Different swab types can be employed because of high separation power, Selective method due to use of variable columns and detectors	Generation of correct results depends on the use of a suitable reference standard, It takes more time to validate this method compared to the others, More expensive, not suitable for products lack a chromophore	Possible interference such as cleaning agent residues interference with the assay must be evaluated. Interferences may lead to changes in retention time, peak height or peak shape, Large amounts of solvent waste has to be disposed. It is essential to extract drugs from their formulations prior to analysis.	HPLC coupled with UV/ Visible is an accurate, precise and robust method for quantitative analysis of pharmaceutical products. Used for both swab and rinse samples, Quantitation of any material produced by degradation
Atomic Absorption	A specific method for both anions and cations in cleaning formulations, Determination of metal residues in drug remaining from the manufacturing process	Limited to residues containing ions.	Cations such as sodium and/ or potassium that may be present in cleaning formulations and also anions, such as the anions from acidic detergents (phosphates, citrates, glycolates) or builders (carbonates, glyconates, silicates, and EDTA (ethylenediamminetetraacetic acid)	Only applicable to metal ions.

			can be measured by this method	
GC and MS	Capable of separation, identification, and quantitation of results, providing an acceptable reference standard is used. Provision of improved peak shape and greater separating power compared to HPLC due to capillary columns usage	Limited to volatile compounds	Some detergent residues are not as volatile as other compounds (such as some solvents). So these agents require to be removed off. The mobile phase does not require disposal.	Mainly used for detection of detergents or solvent residues.
TOC	TOC analyzers are very sensitive	TOC analyzers are not specific enough, therefore should be used with pH and conductivity. Since all of the measured carbon is theoretically attributed to the target substance, the measured amount is the maximum amount of the target calculated according to this assumption. Samples must be water soluble. Swab selection is important due to interference with TOC analysis.	It theoretically measures all the covalently bonded carbon in water based on this assumption that an organic residue contains carbon that can be oxidized under TOC test conditions. Then it measures carbon dioxide. It is typically reported at the part per billion (PPB), or (µg/ml). To reach enough sensitivity quality of water utilized should be high.	It is specific to organic compounds. It is commonly used in the biotechnology industry for cleaning validation. This can also be used for the analysis of detergents, endotoxins, and polyethylene glycol.
pH	Very sensitive to hydrogen ions.	The measurement of pH in unbuffered systems around neutral is unreliable. There is no linear relationship between the level of cleaning agent and pH.	Significant changes in the pH of system can be monitored. However, this technique is not sufficiently capable of measuring actual levels of alkaline or acidic residues.	Trace levels of acids and alkalies used in the cleaning process can be measured by this method.
Conductivity	It can be used as an upper limit estimate of	It is not specific to an ion.	Linear behavior is observed in dilute solutions.	It can be used for cleaning validation

	the amount of an alkaline or acidic cleaning agent.			of cleaning agents.
UV Spectroscoy	Moderately selective, not limited to water as the extraction solution, There is no need of a mobile phase or column, relatively fast method, selection of the swab type is not as restricted as TOC method.	Not quantitative, Not readily applicable to the analysis of mixtures	It can be used for both swab and rinse samples.	It is commonly used for APIs small molecule and detergent residues, as well as, certain surfactants that have a chromophore.
Enzyme-Linked Immunosorbent Assay (ELISA)	Very sensitive and specific for biopharmaceuticals	Expensive, time-consuming. It does not provide enough peak separation. A chromophore is required for specifity.	Its usage is limited due to rapid degradation of proteins by severe conditions of cleaning environment such as temperature and pH.	It is commonly used in the analysis of protein for activities determination
Titrations	It is used to provide the upper level estimates of cleaning agents.	It is not specific enough.	It involves either alkalinity or acidity titrations. More specific titrations are employed to measure components of cleaning agents, such as titrations for chelants in cleaning agents.	It is mainly used for cleaning agent residues since it is very useful for estimating surfactant.
TLC	Suitable for a wide range of materials, Robust and cheap, All components can be observed.	Low sensitivity, not suitable for volatile compounds, It strongly depends on operator skill.	Useful to determine the number of substances generated by materials decomposition which should be investigated during cleaning validation.	Used as a basic identification method.

Table 12: Limits for the microbial status of product contact surfaces [18]

Class	EU GMP Guideline contact plates d=55 (mm) (CFU/plate)	Proposal for implantation Table/ machine (CFU/ 25 cm^2)
A	<1	G: <1, W: 2, A:3
B	5	G: 5, W:2, A:5
C	25	G:25, W:25, A: 50
D	50	G:50, W:100, A:200

G: Guideline value (mean value from ten values in sequence)
W: Alert limit
A: Action limit

Table 13: Sample of the Validation Report (4, 11)

Equipment name:	Therapeutic group:	Test method reference:
Calibrated/validated on:	Limitations of using validated method:	Reference analytical log book:
Location:	Cleaning date:	Limit of detection:
Room number:	Cleaning SOP number:	Next product to be manufactured on the same equipment:
Last product	Revision number:	Safety factor:
Batch number of last product:	Sampling technique:	Recommendations:
Manufacturing date:	Cleaning sample analysis date:	Additional information
Active ingredient :	Assay result:	Any deviation from the protocol:

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