Scientific Research Publishing

American Journal of Analytical Chemistry, 2026, 17(3), 61-82
https://www.scirp.org/journal/ajac
ISSN Online: 2156-8278
ISSN Print: 2156-8251

Cleaning Validation: Analytical Strategies and Practical Implementation Using Chromatographic Methods

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How to cite this paper: Bompelliwar, S.K., Vatsavai, K.R., Kamma, J., Ramgiri, A.K., Meduri, R.T. and Hotha, K.K. (2026) Cleaning Validation: Analytical Strategies and Practical Implementation Using Chromatographic Methods. *American Journal of Analytical Chemistry*, **17**, 61-82.
https://doi.org/10.4236/ajac.2026.173006

Received: March 2, 2026
Accepted: March 27, 2026
Published: March 30, 2026

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Abstract

The cleaning validation is the most important step. Cleaning validation is a crucial aspect of pharmaceutical manufacturing to ensure that residues from previous batches do not contaminate subsequent products. This review explores the principles, regulatory requirements, analytical techniques, validation parameters, challenges, and future trends associated with cleaning validation. The focus is on ensuring compliance with Good Manufacturing Practices (GMP) while maintaining product safety and efficacy. This review paper provides a comprehensive review of analytical method cleaning validation procedures.

Keywords

Analytical Cleaning Validation, MACO Limits, ICH, and FDA Guidance

1. Introduction

Cleaning validation is the documented evidence that approved cleaning procedures effectively and reproducibly remove residues of active pharmaceutical ingredients (APIs), excipients, cleaning agents, microbial contaminants, and endotoxins from manufacturing equipment. With the increasing complexity of drug products and shared manufacturing facilities, robust cleaning validation programs have become essential for regulatory compliance. Effective cleaning validation is no longer viewed as a static regulatory hurdle, but as a dynamic integration

of ICH quality principles. Cleaning validation is a cornerstone of pharmaceutical manufacturing, ensuring product quality and patient safety. The International Council for Harmonization (ICH) guidelines collectively provide a comprehensive framework for designing, executing, and sustaining effective cleaning programs across the product lifecycle. Each guideline contributes a distinct yet complementary perspective to cleaning validation. ICH Q7 establishes the foundation of good manufacturing practices for active pharmaceutical ingredients by defining expectations for equipment cleaning, acceptable residue limits, and thorough documentation. Building on this foundation, ICH Q8 promotes a science and process driven approach to cleaning procedure design, enabling the selection of appropriate cleaning agents, operating parameters, and control points that support scalability and manufacturing flexibility. ICH Q9 introduces a structured quality risk management (QRM) framework, guiding the identification of critical process parameters, prioritization of high-risk equipment and surfaces, and optimization of sampling and analytical strategies. ICH Q10 integrates cleaning validation into the pharmaceutical quality system, emphasizing lifecycle management, traceability, continuous improvement, and periodic review to maintain a state of control. Finally, ICH Q11 aligns cleaning strategies with drug substance chemistry and manufacturing processes, supporting robust analytical method development, practical manual and automated cleaning procedures, and effective management changes as products and processes evolve [1]-[5]. The Cleaning Validation Master Plan serves as the strategic blueprint for navigating this complexity. Rather than evaluating every possible combination, it will utilize a risk-based worst-case logic, prioritizing the most resilient residues based on their chemical solubility and toxicological impact. This framework requires a symphony of expertise: Engineering defines the physical landscape of the equipment, Toxicology sets the biological limits of safety, and Analytical R&D and QC provides the analytical eyes to see what is invisible to the naked eye. Recently, cleaning validation has been one of the most evolving and debated topics and it involved several queries and concerns with respect to practices. The purpose of this document is to provide best practices on the aspects of cleaning validation using the concepts mentioned from regulatory guidance. This review focuses primarily on chemical residue control and analytical verification strategies used in pharmaceutical cleaning validation. While microbiological contamination and endotoxin control may also be relevant in certain manufacturing environments (particularly for sterile and biotechnology products), the primary emphasis of this manuscript is on chemical carryover from active pharmaceutical ingredients (APIs), intermediates, excipients, and cleaning agents. Accordingly, the discussion centers on health-based exposure limits (HBEL), maximum allowable carryover (MACO) calculations, sampling strategies, and analytical techniques commonly applied in cleaning verification, including chromatographic methods such as high-performance liquid chromatography (HPLC) and non-specific analytical approaches such as total organic carbon (TOC). Microbial and endotoxin verification approaches are acknowledged as im-

portant aspects of equipment cleanliness programs but are outside the analytical scope of this review.

2. Regulatory Landscape

The regulatory agencies mandate cleaning validation as part of GMP compliance. **United States Food and Drug Administration (FDA):** 21 CFR Parts 210 and 211 [6]; **ICH Guidelines Q7 and Q9.**

2.1. Risk Assessment and Worst-Case Selection

A risk-based approach is fundamental to cleaning validation. Worst-case scenarios are identified based on factors such as:

- Lowest solubility in cleaning media.
- Highest toxicity or lowest Permitted Daily Exposure value.
- Highest batch size or equipment surface area.
- Most difficult-to-clean equipment.
- Maximum dirty and clean times.

Scientific justification of worst-case selection is critical for regulatory acceptance.

2.2. Acceptance Criteria

Visual Cleanliness: The most basic requirement: equipment must look perfectly clean under high-intensity light.

3. Establishment of Maximum Allowable Carryover (MACO)

Modern cleaning validation approaches rely on toxicological risk assessment to establish scientifically justified residue limits. Several related terms are used within regulatory guidance and toxicological literature. The Permitted Daily Exposure (PDE) represents the maximum amount of a substance that can be safely administered daily without adverse health effects over a lifetime. PDE values are typically derived from toxicological studies using no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) combined with appropriate adjustment factors. The Acceptable Daily Exposure (ADE) is conceptually similar to PDE and represents the daily exposure level considered acceptable based on toxicological evaluation. In many regulatory contexts, PDE and ADE are used interchangeably. The broader concept of HBELs encompasses PDE, ADE, occupational exposure limits, and other toxicologically derived thresholds used to control cross-contamination risks in pharmaceutical manufacturing. In cleaning validation programs, the HBEL value is used to establish the MACO, which defines the maximum quantity of residue from a previous product that may be present in the next manufactured product without posing a patient safety risk. The MACO was established using a risk-based approach in accordance with regulatory expectations and industry's best practices. The calculation methodology was selected based on the availability of pharmacological or toxicological data for the active substance. The MACO will be calculated as follows [7]-[13].

Based on Therapeutic Daily Dose:

When the therapeutic daily dose of both products is known, the MACO is calculated using the following equation.

$$\text{MACO} = \frac{\text{TDD}_{\text{Previous}}}{\text{TDD}_{\text{Next}}} \times \frac{\text{MBS}}{\text{SF}}$$

$\text{TDD}_{\text{Previous}}$ = Therapeutic Daily Dose (TDD) of Previous Product; TDD_{Next} = Therapeutic Daily Dose of Next Product; SF = Safety Factor based on route of administration (Topical: 10-100; Oral: 100-1000; and Parentals: 1000-10000); MBS = Minimum batch size of the next product.

Based on Toxicological Data if TDD is unknown:

If the therapeutic daily dose of the previous product is not available, toxicological data may be used:

$$\text{MACO} = \frac{\text{NOEL}}{\text{TDD}_{\text{Next}}} \times \frac{\text{MBS}}{\text{SF}}$$

NOEL = No observed effect level of previous drug.

General Limit Approach or 10 PPM Criteria:

If the above two approaches result in an impractical or unreasonable MACO value, a general limit approach may be applied.

$$\text{MACO}(\text{ppm or } \mu\text{g}) = \text{MAX}_{\text{CONC}} \times \text{MBS}$$

MACO_{PPM} = MACO ppm Limit

MAX_{CONC} = General limit for maximum allowed concentration of previous substance in the next batch (10 ppm is very frequent)

MBS = Minimum batch size of the next product (kg)

Worst Case Scenario:

A worst-case approach is applied to establish a single MACO value applicable across the entire product portfolio. This unified limit ensures consistent control regardless of individual products.

$$\text{MACO} = \frac{\text{TDD}_{\text{Min}}}{\text{TDD}_{\text{Max}}} \times \frac{\text{MBS}}{\text{SF}}$$

TDD_{Min} = A product with minimum TDD; TDD_{Max} = A product with maximum TDD; SF = Safety Factor; MBS = A product with minimum batch size.

4. Sampling Strategies

Sampling Strategy and Location Selection

Selection of sampling locations is a critical element of cleaning validation because residues are rarely distributed uniformly across equipment surfaces. Regulatory guidance recommends identifying worst-case locations, which are areas most likely to retain residues following cleaning.

Common hard-to-clean locations include:

- Gaskets and seals.
- Valve housings.

- Spray balls.
- Agitator blades.
- Transfer lines.
- Dead legs or poorly drained piping.
- Surface imperfections or rough welds.

Two primary sampling approaches are typically used.

Swab sampling provides direct measurement of residues on defined surface areas and is particularly useful for verifying cleanliness at specific worst-case locations.

Rinse sampling evaluates residues that may remain in inaccessible equipment areas where swabbing is impractical, such as internal piping systems, large vessels, or complex transfer lines.

In many cleaning validation programs, a combination of swab and rinse sampling is used to provide comprehensive coverage of equipment surfaces.

When multiple pieces of equipment are connected in a manufacturing train, results should be interpreted carefully to avoid double counting residue contributions. Cleaning limits are typically established assuming worst-case transfer across the entire equipment train rather than summing residue measurements from each individual component. Recovery studies are required to demonstrate the efficiency of the sampling method. The total MACO for the equipment chain is established, it must be converted into measurable analytical limits for swabbing and rinsing.

Swab Limit calculation:

The swab limit is calculated to determine acceptable residue levels on equipment surfaces:

$$\text{Swab Limit}\left(\frac{\mu\text{g}}{\text{mL}}\right) = \frac{\text{MACO}_{\text{PPM}}(\mu\text{g})}{\text{Equipment Surface Area}\left(\text{cm}^2\right)} \times \frac{\text{Swabbed Surface Area in in}\left(\text{cm}^2\right)}{\text{Swab diluent volume}(\text{mL})}$$

Rinse Limit Calculation:

$$\text{Rinse Limit}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{MACO}_{\text{PPM}}(\text{mg})}{\text{Volume of Rinse Solvent}(\text{L})}$$

Example Calculation: From HBEL to Swab and Rinse Limits

To illustrate the practical application of health-based limits in cleaning validation, a simplified example is provided below.

Assume the following parameters:

HBEL (PDE) for previous product = **5 mg/day**

Minimum batch size of next product = **100 kg (100,000 g)**

Maximum daily dose of next product = **10 g/day**

Step 1: Calculate MACO

The MACO for carryover into the next product can be estimated using:

MACO = (HBEL × Batch Size of Next Product) / Maximum Daily Dose of Next

Product

MACO = (5 mg/day × 100,000 g)/10 g/day

MACO = **50,000 mg**

Thus, a maximum of **50 g of residue** from the previous product could theoretically be present in the entire next batch without exceeding the HBEL exposure limit.

Step 2: Convert MACO to Surface Limit

Assume total shared equipment surface area:

Total surface area = **10,000 cm^2**

Surface residue limit:

Surface Limit = MACO/Total Surface Area

Surface Limit = 50,000 mg/10,000 cm^2

Surface Limit = **5 mg/cm^2**

Step 3: Swab Sampling Limit

Assume:

Swabbed area = **25 cm^2**

Swab recovery = **80%** (Recovery factor = 0.8)

Expected residue on swab:

Residue = 5 mg/cm^2 × 25 cm^2

Residue = **125 mg**

The following two scenarios describe the swab limit adjustment with respect to recovery and reporting of results during the QC testing.

Scenario 1:

The residue limit is not adjusted for recovery (125 mg per swab) when the recovery factor (0.8) is included in the analytical result calculation by dividing the measured response by the recovery factor.

Formula should include:

$$\frac{1}{\text{Recovery Factor}}$$

Result:

$$\frac{\text{Measured}}{0.8}$$

Scenario 2:

The swab limit is adjusted for recovery when the recovery factor is not included in the analytical result calculation. In this case, the lower analytical response due to incomplete recovery is compensated by adjusting the swab limit.

Correcting for recovery:

Adjusted limit = 125 mg × 0.8

Adjusted swab limit ≈ **100 mg per swab**

Result: Measured

Step 4: Rinse Sampling Limit

Assume:

Rinse volume = **1000 mL**

Rinse concentration limit:

Concentration = MACO/Rinse Volume

Concentration = 50,000 mg/1000 mL

Concentration = **50 mg/mL**

This simplified example demonstrates how **toxicological exposure limits can be translated into practical analytical limits used during cleaning verification**.

5. Lifecycle Approach for Cleaning Validation

Cleaning validation typically comprises three interconnected phases: cleaning process design, qualification/validation, and continuous monitoring. Together, these phases ensure that cleaning processes are scientifically justified, reproducible, and capable of maintaining equipment in a consistently clean state throughout routine manufacturing operation. The first phase, **cleaning process design**, focuses on developing a thorough understanding of the manufacturing process and associated equipment to identify potential contamination risks. Risk assessments are conducted to evaluate product attributes, critical process parameters, and equipment surface characteristics that may influence cleanability. Laboratory-scale cleanability and optimization studies are then performed to establish suitable cleaning agents, concentrations, contact times, temperatures, and mechanical actions. The outcomes of these studies support the development of a robust and reproducible cleaning procedure, which is subsequently reviewed and finalized prior to validation. The second phase, **qualification/validation**, aims to demonstrate that the defined cleaning process consistently meets established acceptance criteria under routine operating conditions. A comprehensive cleaning validation plan is developed, incorporating scientific justification for worst-case product and equipment selection. Hard-to-clean locations are identified based on equipment design and process knowledge, and appropriate sampling strategies are selected. Analytical methods used for residue detection are validated to ensure adequate accuracy, precision, sensitivity, and specificity. Following personnel training, validation protocols are executed, and the generated data are critically reviewed and documented in a cleaning validation report. The final phase, **continuous monitoring**, ensures maintenance of the validated state throughout the product lifecycle. This phase includes periodic reviews, routine sampling, trending of analytical data, and ongoing training (**Figure 1**). Observed trends or deviations are evaluated, and cleaning procedures are updated through formal change control processes, supporting continual improvement and sustained regulatory compliance [14]-[16].

6. Common FDA 483 Observations Related to Cleaning Validation

FDA Form 483 observations continue to highlight cleaning validation as a critical area of regulatory focus within the pharmaceutical industry. A review of inspection findings indicates that deficiencies are rarely limited to a single failure but

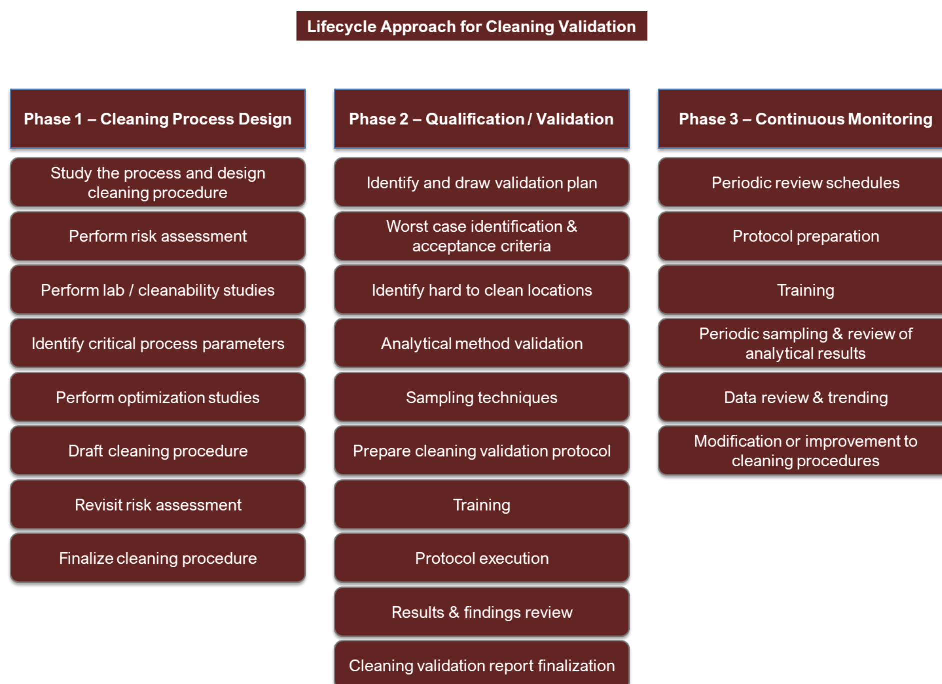

Figure 1. Lifecycle approach for cleaning validation.

instead reflect broader weaknesses in program design, scientific rationale, and quality oversight. One of the most frequently cited observations involves inadequate cleaning validation studies, where worst-case product selection, equipment grouping, or the number of validation runs is not scientifically justified. In many cases, firms fail to demonstrate that selected products represent the most difficult-to-clean or most toxic scenarios. Another common observation relates to the absence of scientifically justified residue limits. Acceptance criteria, including MACO values, are often based on historical defaults or visual cleanliness rather than health-based exposure limits or dose-based calculations. FDA investigators also frequently cite insufficient analytical method validation, particularly when methods lack adequate sensitivity, specificity, recovery, or limits of quantitation aligned with established acceptance criteria. Deficiencies in cleaning procedures and documentation are also prevalent. Cleaning SOPs may lack critical parameters such as detergent concentration, contact time, temperature, equipment disassembly instructions, or defined visual inspection criteria. In parallel, incomplete, or non-contemporaneous cleaning records raise concerns regarding traceability and data integrity. Failure to follow written procedures, inadequate operator training, and weak quality unit oversight further exacerbate these issues. FDA observations also emphasize the importance of lifecycle management of cleaning validation. Firms are often cited for failing to revalidate cleaning processes following changes to products, equipment, cleaning agents, or manufacturing processes. Inadequate change control, lack of periodic review, and insufficient ongoing monitoring undermine the continued validated state. Additionally, poor equipment design, including dead legs and hard-to-clean surfaces, is frequently noted as a con-

tributor to ineffective cleaning.

Examples of FDA 483 Observations

Inadequate Scientific Justification for Residue Limits: A frequent observation (21 CFR 211.67) involves firms failing to provide a scientific rationale for their "Acceptable Daily Exposure" (ADE) or "Permitted Daily Exposure" (PDE) limits. Investigators often find that limits are based on arbitrary values (e.g., 10 ppm) rather than toxicological data [17]. **Failure to Identify Worst-Case Scenarios** FDA Form 483s often cite companies for failing to validate cleaning procedures using "worst-case" products. This includes failing to account for the highest potency, lowest solubility, or most difficult-to-clean formulations in a multi-product facility [18]. **Non-Representative Sampling and Recovery Studies** Observations frequently highlight that swabbing locations are not chosen based on a risk assessment of "hard-to-clean" areas (e.g., valves, gaskets, or agitator shafts). Additionally, firms often fail to perform recovery studies to prove that their sampling method can actually detect residues from specific equipment surfaces [19]. **Lack of Quality Unit Oversight** the Quality Control Unit (QCU) is often cited for failing to review or approving validation protocols and for not investigating cleaning failures or deviations. The FDA emphasizes that "if it isn't documented (and approved), it didn't happen" [20]. **Deficiencies in Continued Process Verification (Stage 3)** Following the 2011 Process Validation guidance, the FDA increasingly cites firms for not having a program to monitor cleaning effectiveness over time. This includes a lack of periodic re-evaluation to ensure that the validated state is maintained as equipment age or as new operators are trained [21]-[23] (**Table 1**).

Table 1. FDA finding examples.

Observation Category	Specific FDA Finding Example	Regulatory Citation
Methodology	Lack of recovery studies for swab/rinse samples.	21 CFR 211.160(b)
Equipment	Use of "visibly clean" as the only criterion without analytical testing.	21 CFR 211.67(b)
Lifecycle	Failure to perform periodic re-validation or trend cleaning data.	21 CFR 211.110(a)
Justification	Inadequate toxicological evaluation for residue limits.	21 CFR 211.100(a)

7. Analytical Methods

Selection of Analytical Techniques for Cleaning Verification

Selection of an appropriate analytical method for cleaning verification depends on several factors, including compound properties, detection limits, and the required level of specificity.

High-Performance Liquid Chromatography with UV detection (HPLC-UV) remains one of the most commonly used analytical techniques due to its robustness, specificity, and suitability for many pharmaceutical compounds that contain UV-absorbing chromophores. However, alternative techniques may be required in

specific situations. HPLC coupled with mass spectrometry (HPLC-MS) offers significantly greater sensitivity and selectivity and is particularly useful when cleaning limits are extremely low due to stringent HBEL requirements [24]. For compounds that lack strong UV chromophores, universal detection methods such as Charged Aerosol Detection (CAD) or Evaporative Light Scattering Detection (ELSD) may be more appropriate. In some cases, Total Organic Carbon (TOC) analysis can be used as a non-specific screening technique to detect residual organic contamination. TOC methods are particularly useful when cleaning agents or residues are difficult to quantify individually, although they lack compound-specific selectivity [25]-[27].

Therefore, the choice of analytical method should be based on:

- Sensitivity required by HBEL-based limits.
- Chemical properties of the residue.
- Potential interference from detergents or excipients.
- Required specificity for regulatory acceptance.

This review article provides a comprehensive overview of HPLC-based cleaning validation [28]-[37] parameters and procedures, drawing on the authors' practical experience and highlighting key considerations for method development and validation.

8. Principles of Cleaning Validation

The primary objectives of cleaning validation include:

- Prevention of cross-contamination.
- Assurance of product quality and consistency.
- Protection of patient safety.
- Compliance with regulatory requirements.

9. Validation Parameters

System Precision is evaluated by repeated injection of a standard solution at 100% of the target concentration six times. The relative standard deviation (%RSD) of the measured response provides a direct measure of the reproducibility of the analytical system under defined operating conditions. High system precision is critical in cleaning validation, where small variations in residual levels may have significant implications for product safety and regulatory compliance.

$$\%\text{RSD} = \frac{\text{Standard Deviation}}{\text{Average Peak Area}\,(n = 6)} \times 100$$

Linearity establishes the quantitative relationship between analyte concentration and detector response. In cleaning validation studies, linearity solutions are prepared at a minimum of five concentrations spanning the quantitation limit (QL) to approximately 200% of the established cleaning limit. Single injections of each solution are analyzed, and a regression line of peak area versus analyte concentration is constructed. Linearity assessment includes calculation of the corre-

lation coefficient (r), evaluation of bias at the 100% concentration level, and determination of the residual sum of squares. Demonstrating linearity ensures that the method can accurately quantify residues across the relevant concentration range encountered during routine cleaning verification.

Limit of Quantitation (LOQ or QL) The LOQ is a critical parameter in cleaning validation to ensure that residual contaminants below regulatory thresholds are accurately detectable. The LOQ represents the lowest concentration at which the analyte can be quantified with acceptable precision and accuracy. To determine the LOQ, linearity solutions are diluted near the expected quantitation limit and analyzed for signal-to-noise ratio (S/N), with six replicate injections performed to confirm method reliability at low concentrations. For QL, a ratio of at least 10:1 is considered acceptable. This QL will also be determined based on the standard deviation linear response and a slope.

$$\text{LOQ} = \frac{10\sigma}{S}$$

where σ = the standard deviation of the response S = the slope of the calibration curve

Limit of Detection (LOD or DL) Accurate determination of LOD is vital for the sensitive detection of trace residues that may impact patient safety or regulatory compliance. defines the lowest concentration of analyte that can be detected but not necessarily quantified with acceptable precision. LOD is similarly determined by diluting linearity solutions near the expected detection limit, evaluating S/N ratios, and performing three replicate injections. For DL, a ratio of at least 3:1 is considered acceptable. This DL will also be determined based on the standard deviation linear response and a slope.

$$\text{LOD} = \frac{3.3\sigma}{S}$$

Method Precision: Method precision in cleaning validation is commonly assessed through **swab accuracy studies**, which evaluate the efficiency and reproducibility of analyte recovery from sampling media under controlled conditions. Prior to use, swabs (Typical swabs Alpha® Polyester Knit TX714A Large Cleanroom Swab, Non-Sterile) are pretreated by placing them into glass beakers containing 200 mL of methanol or acetonitrile and sonicating for approximately 15 minutes. The solvent is then drained, and the procedure is repeated three times. Finally, the swabs are firmly squeezed against the side of the beaker to remove excess solvent, minimize background interference, and ensure consistency. A stock solution of the target analyte is prepared in an appropriate solvent system, typically a hydroalcoholic mixture or a fully organic solvent such as methanol or acetonitrile, selected based on analyte solubility and chemical stability. Defined volumes of the stock solution are evenly dispersed onto the heads of the swabs to simulate contamination. When multiple swabs (typically two) are required for a single sample, the spiked volume is distributed equally across all swab heads to ensure uniform loading. The spiking levels are designed to span approximately 50%

to 200% of the established cleaning limit, with typical target concentrations at 50%, 100%, and 200%. Samples are prepared in triplicate at each level, while the 100% cleaning limit level is evaluated using six independent replicates to support statistical assessment of method precision. Following spiking, swabs are transferred into appropriate extraction vessels and subjected to a controlled drying step in an oven at 40°C for 15 minutes to simulate real-world residue drying conditions. After cooling to ambient temperature, a fixed volume of extraction solvent (e.g., 10.0 mL) is added to each test tube. The samples are then sonicated for 10 minutes and mixed thoroughly using a manual vortex mixer to maximize analyte extraction for about 2 minutes. Clear extracts are transferred to HPLC vials and analyzed. Swab blank solutions are prepared by exposing unused swabs to the extraction solvent under conditions identical to those used for sample collection. Analyte recovery is calculated as the ratio of the measured concentration to the theoretical spiked concentration, expressed as a percentage. Recovery data provide a quantitative measure of swab efficiency and method precision, which are critical for ensuring the reliability of residue quantification during routine cleaning verification.

$$\text{\% Recovery} = \frac{\text{Amount Found}\left(\mu\text{g/mL}\right)}{\text{Amound Added}\left(\mu\text{g/mL}\right)} * 100$$

Surface Accuracy and Quantitation Limit Recovery: Surface accuracy studies are conducted to evaluate the recovery of residues from representative product contact surfaces, most commonly 316 stainless steels plates. Stainless steel plates with a defined roughness are cleaned using water followed by methanol and allowed to air-dry prior to use. A stock solution of the analyte is prepared in a suitable solvent system, consistent with that used for swab accuracy studies. Known volumes of the stock solution are evenly applied to a defined surface area of the plate using a gas-tight syringe. The typical recovery study concentrations include the quantitation limit (QL), 100%, and 200% of the cleaning limit, thereby covering the lowest reportable concentration through worst-case residue scenarios. The area designated for sampling is maintained at 100 cm^2 (4 × 4 inches) to ensure consistency across studies and alignment with regulatory expectations. After application, the plate is allowed to air-dry and is visually inspected for evidence of residual material, with observations documented. Swabbing is performed using a standardized multidirectional technique to ensure complete surface coverage. The first swab is swabbed horizontally from left to right and then from right to left using opposite sides of the swab. A second swab is swabbed vertically from top to bottom and then bottom to top, again using opposite sides (**Figure 2**). This approach maximizes physical contact between the swab and the surface, improving recovery robustness. Surface blank solutions are generated by swabbing representative surfaces using the same number and type of swabs, but in the absence of the product. Following sampling, swabs are subjected to the same drying, extraction, sonication, and mixing procedures as described for swab accuracy studies. If recovery at the QL level does not meet predefined acceptance criteria, accuracy

studies are repeated at the next higher concentration level to confirm method suitability near the lower limit of quantification. While stainless steel remains, the primary surface evaluated during cleaning validation, manufacturing equipment often incorporates additional materials such as anodized aluminum, polyester plastics, acrylic glass, polytetrafluoroethylene (Teflon), and silicone rubber (Figure 3). Validation of these alternative product contact surfaces is dependent on the specific equipment train used in manufacturing operations. The manufacturing team identifies applicable surface materials, which are then incorporated into the analytical method validation strategy to ensure comprehensive coverage of all relevant product contact interfaces.

$$\% \text{ Recovery} = \frac{\text{Amount Found}(\mu g/mL)}{\text{Amound Added}(\mu g/mL)} * 100$$

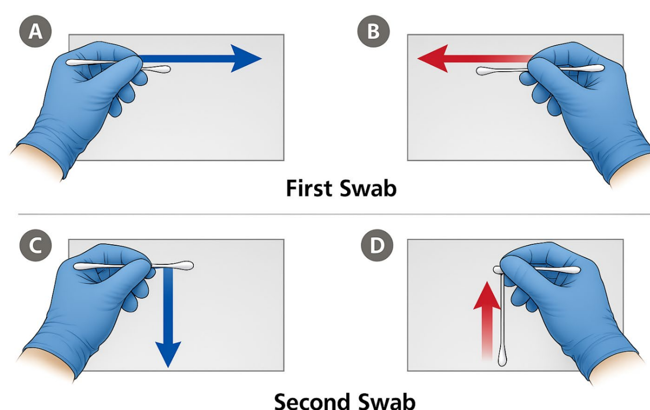

Figure 2. Typical swabbing procedure.

Figure 3. Different Cleaning Validation Plates with size of 4 × 4 inches.

Intermediate Precision Intermediate precision assesses the reproducibility of the analytical method under normal but variable operating conditions. This eval-

uation is typically performed by a second analyst on a different day, using a separate HPLC system and chromatographic column. Intermediate precision studies encompass system suitability testing, swab accuracy at 100% of the cleaning limit (six replicates), and surface accuracy studies from stainless steel plates conducted in triplicate at the QL, 100%, and 200% levels. Demonstrating consistent performance across analysts, instruments, and days provides assurance that the analytical method is robust and suitable for routine cleaning validation applications in a regulated pharmaceutical environment.

Specificity A robust analytical evaluation relies on the careful preparation of swab blanks and surface blanks, which serve as essential controls for method accuracy and specificity. These controls account for potential analytical contributions from the sampling material and procedural handling, thereby enabling precise differentiation between true residues and background noise. To assess method performance and recovery, placebo and detergent solutions are prepared in the extraction solvent. Comparative analysis of the diluent, swab blanks, surface blanks, placebo, and detergent (Clean in place 100: CIP-100) solutions allows for rigorous evaluation of analytical specificity, sensitivity, and robustness. This approach ensures that the analytical method can reliably detect residual contaminants, support regulatory compliance and maintain product quality. Implementing such structured control strategies is widely recommended in the literature as a best practice for cleaning validation, providing confidence in the accuracy of residue quantification, and supporting the integrity of pharmaceutical manufacturing processes. Prepare a swab blank solution and swab blank solution after swabbing surface (Surface Blank for respective surfaces) is using the same number and type of swaps. Prepare placebo and detergent in extraction solvent at an appropriate concentration as per the protocol. Analyze the diluent, swab blank, surface blank, placebo solution, and detergent.

Method Range: The analytical method range is a critical performance characteristic that defines the interval over which the method demonstrates acceptable accuracy, precision, and linearity for residue quantification in cleaning validation. Unlike conventional drug product assays, cleaning validation methods must reliably quantify analytes at trace levels and across a wide concentration span relative to the established cleaning limit. Consequently, method range is commonly established using **surface accuracy (recovery) data**, as this approach directly reflects real-world sampling and extraction conditions. Regression analysis is performed by plotting detector response (peak area) versus analyte concentration using data generated from surface accuracy studies. The evaluated concentration range typically extends from the quantitation limit (QL) through approximately 200% of the cleaning limit, thereby encompassing both minimum reportable levels and worst-case residue scenarios. Linear regression parameters, including the correlation coefficient (r), are calculated to assess proportionality between response and concentration. In addition, bias at the 100% cleaning limit level is evaluated to confirm method accuracy at the critical decision point. Establishing method range

using surface recovery data ensures that analytical performance is representative of routine cleaning verification activities and supports scientifically justified acceptance criteria.

Solution Stability of Standard and Sample: Solution stability studies are conducted to demonstrate that analytical results remain reliable over the time required for sample preparation, storage, and analysis. Both standard solutions and extracted sample solutions are prepared and stored under defined conditions, most commonly at ambient laboratory temperature on the benchtop. Stability is assessed by analyzing the solutions at the initial time point and at predefined intervals thereafter. Stability is expressed as the percentage difference between the initial analytical response and the response obtained at each subsequent time point. When the same system is used throughout the study, percent difference for the standard and test solutions is calculated based on peak area or reported result comparisons relative to the initial measurement. When different systems are employed, normalization to standard weight is applied to account for variability introduced by instrument-specific response factors. If stability acceptance criteria are not met at a given time point, the study is repeated using shorter evaluation intervals or under refrigerated storage conditions. This tiered approach ensures that sample handling conditions are appropriately controlled while maintaining operational flexibility within quality control laboratories. Demonstration of solution stability supports method robustness and ensures data integrity during routine cleaning validation testing.

$$\%\ \text{Difference for Standard} = \frac{\text{Abolute}\left(\text{Initial Peak Area} - \text{Stability Point Peak Area}\right)}{\text{Initial Peak Area}} * 100$$

$$\%\ \text{Difference for Sample} = \frac{\text{Abolute}\left(\text{Initial Result} - \text{Stability Point Result}\right)}{\text{Initial Result}} * 100$$

Swab Stability and Holding Time Evaluation Swab stability studies evaluate the impact of holding time between sample collection and analysis on analyte recovery. These studies are particularly important in cleaning validation, where logistical constraints may necessitate delayed analysis following surface sampling. To assess swab stability, the analyte is spiked directly onto the swab head at the 100% target concentration level. For each time point, samples are prepared in triplicate to support statistical evaluation. Spiked swabs are stored in appropriate extraction vessels at room temperature on the benchtop, and the elapsed time is recorded from the moment of spiking. At predefined intervals, swabs are extracted and analyzed as described in the precision section, and recovery is calculated relative to the theoretical spiked amount. Recovery results are compared against predefined acceptance criteria to confirm analyte stability on the swab matrix over time. If recovery falls outside acceptable limits at a given time point, the study is repeated using shorter holding times or refrigerated storage conditions. Establishing swab stability and allowable holding times is essential for ensuring consistent

residue recovery and for defining operational controls within cleaning validation programs. A pictorial representation of the cleaning validation parameters is provided in Figure 4, while the acceptance criteria are presented in Table 2.

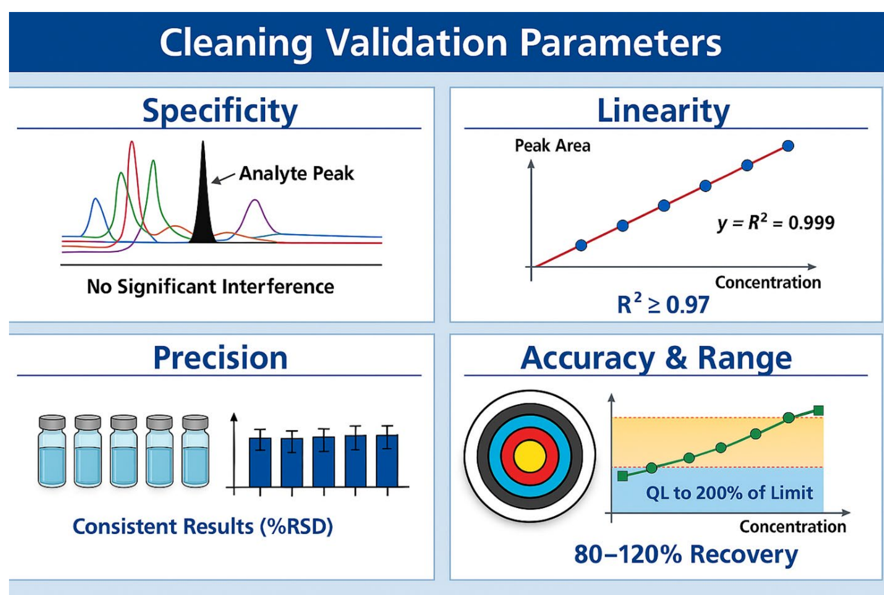

Figure 4. Pictorial representation illustrating the cleaning validation parameter.

Table 2. Analytical cleaning validation parameters and acceptance criteria.

Parameter	Acceptance Criteria
System Precision	The %RSD obtained from six replicate injections of the standard solution should be no more than (NMT) 10.0%.
Linearity	The correlation coefficient (r) of peak area versus analyte concentration should be not less than (NLT) 0.97. Bias at the 100% cleaning limit level should be NMT ±5.0%.
LOQ	The QL concentration should provide an average signal-to-noise (S/N) ratio of 10–20. The %RSD obtained from six replicate QL injections should be NMT 15.0%.
LOD	The DL concentration should provide an average signal-to-noise (S/N) ratio of 3–10.
Method Precision	The mean recovery should be between 80.0% and 120.0%. The %RSD of recovery at each concentration level should be NMT 10.0%. A recovery factor should be applied when recovery is less than 80.0%.
Surface Accuracy and QL Recovery from Plate	The mean recovery should be between 50.0% and 120.0%. The %RSD of recovery at each level should be NMT 10.0%. Recovery factors should be applied when accuracy is less than 80.0%.
Intermediate Precision	Mean swab recovery should be between 80.0% and 120.0%, with %RSD NMT 10.0%. The difference between mean values obtained from method precision and intermediate precision studies should be NMT 15.0%. Mean surface recovery should be between 50.0% and 120.0%, with %RSD NMT 10.0%. Recovery factors should be applied when accuracy is less than 80.0%.
Specificity	Interference from diluent, swab blank, surface blank, placebo, and detergent solutions should not exceed 2.0% of the analyte response at the target concentration.
Range	The correlation coefficient (r) of peak area versus concentration should be NLT 0.97, and bias at the 100% level should be NMT ± 5.0%.
Standard Stability	Agreement between the initial and stability point standard responses should be within ± 10.0%.

Continued

Sample Stability	Sample results at each stability time point should be within ± 10.0% of the initial result.
Swab Stability	Mean recovery should be between 80.0% and 120.0%, with %RSD of recovery results at each level NMT 10.0%.

Note: The acceptance criteria mentioned above table based on the author's experience. The wider criteria are acceptable if specifications or results are justified.

10. Typical QC Testing Method Practice

Prepare the mobile phase and diluent as per the validated method. Pre-treat the swabs by immersing them in 100% methanol or acetonitrile (ACN). Sonicate the swabs for 15 minutes, then drain and discard the solvent. Repeat this pre-treatment procedure three additional times. After pre-treatment, provide the swabs in empty test tubes to Manufacturing or QA personnel for equipment swabbing. Following swabbing, return the used swabs to their respective test tubes. Place the tubes in an oven at 40 °C for 15 minutes to evaporate residual solvent. Allow the tubes to cool to room temperature. Once cooled, add 10 mL of diluent to each test tube. Sonicate the samples for approximately 10 minutes, then mix thoroughly using a manual vortex for 2 minutes to ensure complete extraction (**Figure 5**).

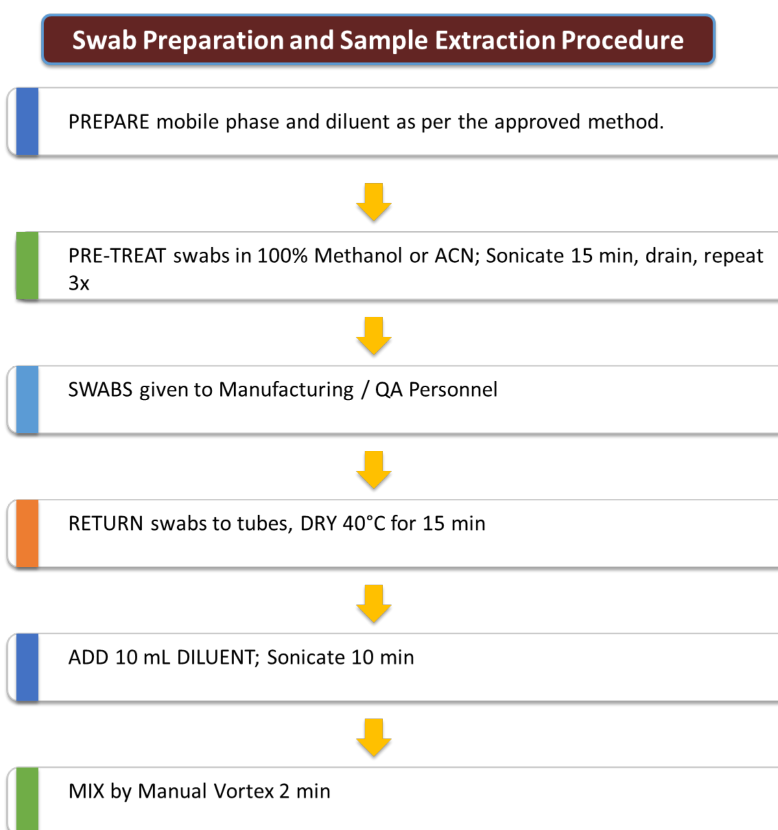

Figure 5. Typical quality control testing systematic diagram.

Typical QC testing will calculate the cleaning content as follows.

$$\text{Content}\left(\mu\text{g/mL}\right) = \frac{\text{Sample Area}}{\text{Average Standard Area}} \times \text{Standard Concentration}\left(\frac{\text{mg}}{\text{mL}}\right) \times \frac{\text{Potency}}{100} \times \frac{1}{\text{Recovery Factor}} \times 1000\left(\frac{\mu\text{g}}{\text{mg}}\right)$$

$$\text{Cleaning Limit}\left(\mu\text{g}/100\ \text{cm}^2\ \text{swab area}\right) = \text{Content}\left(\mu\text{g/mL}\right) \times \text{Sample Dilution}\left(\text{mL}\right)$$

11. Challenges

Sometimes, developing a cleaning verification method for low-potency compounds can be challenging. In such cases, wider acceptance criteria may be required to ensure the method is practical and scientifically justified. Additionally, for molecules that lack chromophores, it may not be feasible to develop a detection method using conventional UV-based techniques. In these situations, alternative analytical approaches such as Total Organic Carbon (TOC) analysis should be considered. Sometimes, the selection of an appropriate method depends on the compound's physicochemical properties, sensitivity requirements, and overall risk assessment. The development of HPLC methods for cleaning verification presents several technical and scientific challenges, particularly in the context of increasingly stringent regulatory expectations and complex drug substances. One of the primary challenges is achieving adequate sensitivity to meet (HBEL derived acceptance criteria [38] [39] [40]. Low-potency or highly potent compounds often require detection limits at trace or sub-trace levels, which may exceed the practical sensitivity of conventional HPLC-UV methods. This limitation is further compounded when analytes exhibit weak or no chromophore properties, making UV detection unsuitable and necessitating alternative detection strategies or orthogonal methods. Matrix interference from cleaning agents, excipients, or equipment surface residues can also complicate method development. Swab and rinse samples often contain residual detergents or extractables that may co-elute with the target analyte, impacting specificity, peak purity, and quantitation accuracy. Careful selection of chromatographic conditions and sample preparation techniques is therefore critical to ensure method selectivity. Recovery variability represents another significant challenge, particularly in swab-based sampling. Differences in surface materials, swabbing techniques, and solvent extraction efficiency can lead to inconsistent recoveries and increased method variability. Demonstrating acceptable and reproducible recovery across representative equipment surfaces is often resource-intensive and may limit method robustness. Method robustness and ruggedness are further challenged by the need to balance sensitivity, run time, and system suitability requirements. Minor variations in mobile phase composition, column chemistry, or instrument performance can have a pronounced impact on low-level quantitation. As a result, extensive robustness studies are required to ensure method reliability during routine cleaning verification.

12. Future Trends

Current trends in cleaning validation reflect a shift toward more science and risk-based approaches. There is increasing adoption of HBEL based limits to establish acceptance criteria that are toxicologically justified and aligned with patient safety considerations. This approach moves away from legacy dose or default-based limits and allows for more compound-specific and defensible cleaning strategies. In parallel, QRM principles are being more fully integrated into cleaning validation programs to systematically assess and control the risk of cross contamination. Risk based tools are increasingly used to evaluate factors such as product potency, equipment design, cleaning process capability, and analytical method performance, enabling resources to be focused where the potential risk is greatest. Advances in technology have also influenced modern cleaning verification practices. The expanded use of Process Analytical Technology (PAT) tools and automated cleaning systems has improved real time monitoring, process consistency, and overall control of cleaning operations. These technologies reduce operator dependence, enhance reproducibility, and support a more data driven approach to cleaning verification. Finally, there is a growing emphasis on lifecycle based and continuous cleaning verification approaches. Rather than treating cleaning validation as a one-time activity, manufacturers are increasingly adopting ongoing verification strategies to ensure sustained cleaning effectiveness throughout the product and process lifecycle. This approach supports continual improvement and helps maintain compliance in the face of process changes and evolving regulatory expectations.

13. Conclusion

A robust, science and risk-based cleaning validation program is essential for ensuring pharmaceutical product quality and regulatory compliance. Continued evolution of regulatory expectations highlights the importance of toxicological assessment, lifecycle management, and ongoing performance monitoring.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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