# A CRO's Perspective on Cleaning Validations

Eric A. Lingenfelter, MBA
Thomas C. Lehman, Ph.D.

Lancaster Laboratories
Method Development and Validation Department

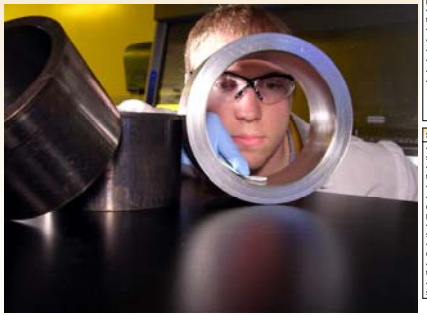
## Abstract/Introduction

Pharmaceutical manufacturing equipment must be properly cleaned to ensure the removal of product residue, cleaning chemical residue and microbes prior to manufacturing. Cleaning methods need to be developed and validated to prevent the risk of cross contaminated and adulteration of products. These methods need to be validated to confirm that the defined cleaning process sufficiently removes potential contaminants. Establishment of method limits and selection of the appropriate cleaning techniques and detection methods are critical to prove that the defined method conditions effectively clean the manufacturing equipment. Validation of the defined method conditions provides confidence in the defined method conditions. This poster will discuss the selection of the appropriate cleaning procedure, including selection of various sampling techniques, and the detection options available to monitor the amount of residual contaminant. The use of the appropriate tests during method validation are critical in proving that the method performs as intended, and these validation tests are discussed as well. In addition, revalidation of analytical techniques and the use of correction factors will be discussed.

## Establishment of Appropriate Limits

In the US FDA Guide to Inspections Validation of Cleaning Processes, it is stated, "The firm's rational for the residue limits established should be logical based on the manufacture's knowledge of the materials involved and be practical, achievable, and verifiable." The FDA gives examples of analytical detection levels such as 10 ppm, biological activity levels such as 1/1000 (0.1%) of the normal therapeutic dose and organoleptic levels such as no visible residue.[1] One can easily apply these examples to determine the amount of allowable carry-over of product residues.

- No more than 10 ppm of any product should appear in another product.
- No more than 0.1% of the normal therapeutic dose of any product will appear in the maximum daily dose of the following product.
- No amount of product residue should be visible on the surface of the equipment after the cleaning procedure has been performed.



## DISCUSSION

### Visual Determination of Residue

The FDA states, "When the cleaning process is used only between batches of the same product (or different lots of the same intermediate in a bulk process), the firm need only meet a criteria of 'visibly clean' for the equipment."

- Enhance visual detection by setting up spiking studies
- Spike coupons with different known amounts of residue
- Training personnel observe coupons to determine at which level coupon appears clean
- Acceptance level set at highest level spiked coupon that appears clean[2]

**Disadvantages to Visual Inspection**

- Too many variables that can influence results
- Coupons must be observed in exact same viewing conditions as equipment in the field
- Not all equipment can be viewed similar to coupon sitting on lab bench
- The lighting must be the same
- The angle of viewing must be the same
- The distance the viewer is from the surface must be the same
- Observer may only be able to see edge of stain instead of the 'body' of the stain itself[3]
- Results are not quantitative

### Selection of an Appropriate Extraction Solution

- Decision should be based on the solubility of the residue
- Typical extraction solutions utilized include alcohols, waters, buffers or combinations of the three solutions

### Rinse or Swab Sampling?

The sampling technique selected must be capable of quantitatively determining the amount of residual material on the manufacturing equipment. Many considerations go into making the decision on the sampling technique including

- Ease of access for sampling
- Size of equipment
- Solubility characteristics of compound of interest

**Rinsing**

- Applicable for small surface areas, and difficult to reach areas where traditional swabbing procedures may be difficult
- Surface should be rinsed long enough to ensure complete coverage and sufficient removal of the target residue.
- More simplistic than swabbing procedures
- Sample is generally collected from final water rinse of equipment

**Swabbing**

- Ideal for residues not easily removed with water rinsing
- Swabs can physically remove insoluble residues
- Chosen swabs must exhibit three important characteristics:
  1) Ability to recover desired residue from given surface
  2) Ability to release the reside into an extraction solvent for analysis
  3) Must not contribute excessive interference or background during analysis

### Swabbing Techniques

- Prior to swabbing, swabs are soaked for a few minutes in a vial with the extraction solution to saturate the swab head
- Excess solution is removed from the swab head by gently pressing the head on the inside of the vial
- The prepared swabs are utilized to swab the appropriate area using various swabbing patterns
- Common patterns use partially overlapping parallel strokes in one direction or opposite directions back-and-forth. The swab head is then flipped to the other side, and the same pattern is repeated at right angles to the first **(see Figure 1.A)**.
- Another variation is to perform overlapping zig-zag strokes in opposite directions, ensuring the swab head never leaves the surface being evaluated. **(see Figure 1.B)**.
- The swab head is placed back into the vial by clipping the handle above the head with a clean cutting tool.
- One swab may be sufficient to remove residue, but a second or even a third swab can be used to repeat the swabbing pattern to increase the recovery of the residue.
- Depending on the extraction solution, it may be advantageous to use a dry swab after the wet swab to ensure any remaining solution on the coupon is collected.

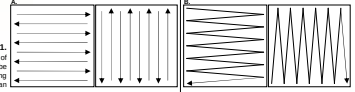



**Figure 1.** There are a variety of patterns that can be followed when using swabs to clean

### Methods of Detection: There are multiple detection options for cleaning validation/verification samples producing quantitative information on residues.

| Instrumentation | Benefits | Drawbacks |
|---|---|---|
| **IMS – Ion Mobility Spectrometry**<br>•Characterizes chemical substances based on their gas-phase ion mobilities<br>•Provides detection and quantitation of trace analytes<br>•Utilizes atmospheric pressure chemical ionization (APCI) – a soft ionization technique that produces molecular weight information | •Ultra-fast quantitative analysis (~30 seconds per sample)<br>•Sub-nanogram sensitivity<br>•The ability to analyze a broad range of compounds with no chromophore needed<br>•No mobile phases, columns or vacuum is required for operation<br>•Sample introduction via either thermal desorption or by high performance injection | •Compounds must be vaporizable and ionizable for IMS detection to be used.[4]<br>•Samples must be relatively clean<br>•Ultra pure extraction solutions should be used<br>•Not suitable for multiple component matrices |
| **TOC – Total Organic Carbon**<br>•Analysis is specific to organic compounds<br>•Theoretically measures all the covalently bonded carbon in water[5] | •TOC detects organic residues or contaminants<br>•Non-specific detection<br>•TOC methods are easy to develop and relatively inexpensive to perform | •TOC analysis incorporates all organic molecules in solution<br>•Result may comprise carbon from various components and not just compound of interest[5]<br>•Contaminating material(s) need to be organic and contain carbon that can be oxidized under TOC test conditions[5,6]<br>•Samples must be water soluble (above MCL)<br>•Sensitive to interferences |
| **UV-Visible Spectophotometry**<br>•Commonly used for detection of small molecule active pharmaceutical ingredients or detergent residues for swab and rinse samples | •Not limited to water as the extraction solution<br>•Provides quantitative results<br>•No mobile phase or column required<br>•Fast spectral acquisition<br>•Larger swab selection can be used compared to TOC | •Lacks peak separation<br>•Chromophore required for specificity |
| **HPLC - High Performance Liquid Chromatography**<br>•Used for detection of small molecule active pharmaceutical ingredients or detergent residues for both swab and rinse samples allowing for separation of multiple components | •Not limited to water as the extraction solution<br>•Provides separation of multiple components and allows identification of specific peaks of interest and quantitative results provided a suitable reference standard is used<br>•Multiple detection options available (UV, Photodiode Array, Fluorescence, Refractive Index, Evaporative Light Scattering (ELSD), Corona Charged Aerosol (CAD) detectors, etc.)<br>•Large variety of swab types can be used due to separation power<br>•HPLC in tandem with mass spectroscopy (MS) offers selectivity while separating the API from its degradents based off mass-to-charge ratio of the compound of interest.[4] | •May require more development and validation time when compared to other forms of detection depending on current information about the API and excipients being used in the formulation.<br>•HPLC/MS analysis will have increased cost |
| **GC and GC/MS – Gas Chromatography and Mass Spectroscopy**<br>•Used mainly for detection of detergent residue<br>•Specific to volatile and semi-volatile organic compounds | •Improved peak shape over HPLC due to capillary column usage<br>•Provides separation of multiple components and allows identification of specific peaks of interest and quantitative results provided a suitable reference standard is used<br>•GC in tandem with mass spectroscopy (MS) offers selectivity while separating the API from its degradents based off mass-to-charge ratio of the compound of interest | •Samples require vaporization |

### Summary of Typical Validation Components[7]

| Validation Component | Description | "Typical" Acceptance Criteria |
|---|---|---|
| **Accuracy** | Accuracy should be assessed at a minimum of 3 concentration levels, each prepared in triplicate. Typically performed with concentrations ranging from 80 to 120% of final theoretical concentration. Accuracy may include evaluate of recover from spiked swabs or from spiked surfaces. | •Swab recovery: mean recovery of 90% - 110% theoretical. %RSD ≤ 10%<br>•Surface recovery: mean recovery of 85% - 115% theoretical. %RSD ≤ 15% |
| **Precision** | Precision is frequently performed in conjunction with Accuracy. For precision the 100% spike level (whether swabs or surfaces) is prepared as 6 replicates. These 6 replicates are prepared by 2 separate analysts and the results are compared. | •All system suitability meets method criteria.<br>•Intermediate precision data and precision data (combined) must have an RSD of ≤ 15%. |
| **Linearity** | Performed using a minimum of 5 concentration levels, each injected in duplicate. Linearity may range from the limit of quantitation up to 200% of the MCL. | Correlation coefficient (r) ≥ 0.995 or coefficient of determination ($r^2$) ≥ 0.99. |
| **Specificity** | Typically both swabs and surfaces are evaluated to determine if interferences with the compound of interest are present. | Detected analyte of interest must not exceed 10% of the mean MCL or have a S/N ≥ 10. |
| **LOD** | Prepare standard solutions at the estimated LOD (3 preparations) and analyze. | Analyte of interest must be detected in all injections and be in the retention time region of the peak of interest (~ S/N of 3). |
| **LOQ** | Prepare standard solutions at the estimated LOQ (3 preparations injected in duplicate) and analyze. | Analyte of interest must have a recovery of 75% - 125% of theoretical and have an RSD of ≤ 20% (~S/N of 10). |
| **Robustness** | The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. | The varied condition must meet the system suitability requirements outlined in the analytical method. |
| **Stability** | The ability of a standard or sample preparation solution to meet method specifications over time. | Recovery value of 98.0% - 102.0% when compared to fresh standard solutions. |

### Is TOC the Answer?

Historically, if a compound was rendered by the Merck Index to be only slightly water soluble, everyone immediately ruled out the option of TOC. Lancaster Laboratories has developed methods utilizing TOC for compounds that are not fully water soluble. In order to be TOC compliant, compounds only need to be slightly soluble in water (for example, if a maximum contamination limit (MCL) is set to 10 ppm, the compound only needs to be soluble at 11 ppm in water to be measured in the range of interest). This is a very interesting concept that is growing in popularity across the industry.

**Here are some key factors to evaluate when determining if TOC is the correct approach for a cleaning validation:**

- Is the carbon content of the sample great enough to be detected once the sample is appropriately diluted?
- Is the compounds solubility in water above the concentration of the desired MCL?
- Does the method need to be compound specific?
- Can acceptable surface recoveries be achieved by rinsing with water or swabbing with water, a weak acid or base?

### Application of Correction Factors

In some cleaning validation studies, it may be determined that not all the residue on the surface can be removed, thus resulting in low recoveries. To increase these results, the application of recovery factors may be necessary. The following is a list of considerations one should evaluate if correction factors are deemed appropriate.

- Recovery factors are usually not applied if recovery results are above 70%, however, there is no standard limit
- Recovery factors must be set under sound scientific justification
- Recovery factors should not be used if recoveries are too low (For example, if recoveries are consistently around 10%, a 90% recovery factor would not be appropriate.)
- Recovery factors need to be set prior to or during validation – not during routine monitoring
- All results used to determine the recovery factor need to be consistent and reproducible.
- Method optimization should always be explored as an alternative prior to using recovery factors

### How to Handle Failing Data

- The best way to approach this issue is to address it before it is an issue.
- Dedicate a section of the cleaning validation master plan or protocol to handling failing data
- Lay out a step by step investigation of the results in advance, thus not influencing decisions made on instance by instance circumstances
- Regulatory agencies like to see that failing results were handled in a consistent and systematic manner
- All data not meeting acceptance criteria should be handled as a deviation where data should be first verified, resolved, and finally approved
- Samples may need to be retested or more samples may need to be collected to verify outlying results
- Modification to the method, protocol, SOP or master plan may need to be entertained if results indicate that a criteria or limit is not attainable
- All of these scenarios should be investigated during the feasibility/method development/validation stage of the cleaning validation study

### When do you need to Revalidate a Cleaning Validation Method?

- Cleaning validation procedures should be revalidated when the equipment train of the manufacturing process is changed. Possible changes in the equipment train include the surface type utilized and/or surface area, which can lead to the establishment of a new maximum contamination limit. Usually a full validation can be avoided and only certain elements of the cleaning validation need to be revalidated. If the new limit is within the previously established linear range, only surface recoveries (bracketing the new limit) and surface residue specificity would need to be revalidated. These same two elements would need to be revalidated if a surface type were changed. If the new limit is outside the previously established linear range, linearity would need to be extended above or below the new limit and swab recovery, surface recovery, and surface residue specificity would need to be revalidated. For a new limit below the established linear range, a new standard concentration at this level may be recommended. However, if the existing method is not linear through the new level, a new standard concentration would be necessary, and this would required full revalidation.
- Other possible but less likely reasons to revalidate swab recovery, surface recovery, and surface specificity would be a change in the type of swab or swabbing pattern. For a change in swab type, swab specificity would also need to be revalidated. For any of the previously listed changes, elements that would not require revalidation are limits of detection and quantitation and linearity.
- The prior revalidation discussion assumes that the validated method was for swab samples and not rinse samples. For rinse samples, validation elements involving swabs and surfaces would not need conducted. Additionally, any changes in the synthesis of the drug substance, changes in the composition of the finished product, or changes in the analytical procedure would require revalidation according to ICH guidance[7].

## CONCLUSIONS

Establishment of an appropriate cleaning validation platform is critical in any manufacturing process. There are many options to choose from when establishing the cleaning program and care must be taken to ensure that the sampling technique, and analytical monitoring methodology selected, along with establishing the appropriate limit (MCL) and compliance of the approach meet the requirements and the intent of the sample program.

References:

1. Rebecca Brewer Presentation IVT Chicago, (July 2007)
2. FDA: Guide to Inspections Validation of Cleaning Processes (1993)
3. D.A. LeBlanc, "Establishing Scientifically Justified Acceptance Criteria for Cleaning Validation of finished Drug Products," Pharm. Technol. October 1996.
4. Cleaning Validation and Critical Cleaning Processes, Dober Research Works
5. TOC Applications in Pharmaceutical Cleaning Validation, Jon Yourkin GE Analytical Instruments, General Electric Powerpoint presentation
6. Pharmaceutical Technology (April 2005), Using Visible Residue Limits for Introducing New Compounds into a Pharmaceutical Research Facility
7. ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2(R1), November 2005