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Analytical Methods for Cleaning Validation

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ABSTRACT

Residue identification in a pharmaceutical manufacturing environment involves; the cleaner, primary ingredients, excipients, decomposition products, and preservatives. This document is intended to help with the cleaner residue identification. Residue detection method selection for cleaners can involve specific methods for specific cleaner ingredients such as; high performance liquid chromatography (HPLC), ion selective electrodes, flame photometry, derivative UV spectroscopy, enzymatic detection and titration, or it can involve non-specific methods that detect the presence of a blend of ingredients such as: total organic carbon, pH, and conductivity. The FDA prefers specific methods, but will accept non-specific methods with adequate rationales for their use. For investigations of failures or action levels, a specific method is usually preferable.

INTRODUCTION

There are a variety of analytical methods that can be chosen to measure target residues. This article will cover analyhcal methods for chemical residues. The selection of an analytical method for measuring residues is closely related to the chemical nature of target residues and to the analytical limits established for those residues. Chemical nature includes whether the target residue is organic or inorganic, is soluble in water or other solvents, its degree of polarity, and its stability in the cleaning environment. A key element in the selection of an appropriate analytical method is that the method produces a result that has a logical, scientific link with the target residue [l, 2,3] **.** For example, if the target residue is an organic, nonionized drug active (XYZ), and the acceptance criterion is 2 ppm in the analyzed sample, then using conductivity as an analytical tool would be inappropriate because there is no scientific relationship between the presence of the target residue in the analytical sample and the measurement of conductivity in the test sample. A high performance liquid chromatography (HPLC) method, which was

validated to measure XYZ at appropriate levels, would be an acceptable method to choose a s an analytical tool for cleaning validation studies. One can go several steps further, however, and consider conditions under which that HPLC method would be inappropriate for residue testing for validation purposes. For example, if there was evidence that XYZ was degraded during the cleaning process, then that specific HPLC method may not be appropriate for analyzing the target residue. If the HPLC procedure were used for either a swab sample or rinse water sample analysis, the results most likely would be below the detection limit of the method. This would not be helpful information, because if the cleaning or rinsing processes were inadequate, then the species that would be left behind would be the degradation product of XYZ, not *XYZ* itself.

DETECTION LIMITS

The Food and Drug Administration (FDA) cleaning validation guidelines call for companies to "determine the specificity and sensitivity of the analytical method used" **[3].** *Sensitivity* at one time was a useful word for analytical methods (referring to the slope of the working curve); however, in popular usage, it has been loosely used and has become synonymous with either "limit of detection" (MD) or "limit of quantitation" (LOQ). The FDA is referring to LOD/LOQ: The LOD/LOQ of the analyhcal method should be at or (preferably) below the acceptance criterion in the analyzed sample. If the target limit in the analytical sample were 5.2 ppm, and a method was only able to detect down to 10 ppm, that method would not be useful for cleaning validation purposes. Because most pharmaceutical manufacturers like to have significant safety built into their processes, they would generally prefer an analytical method with an LOD of at least 25 percent of the target residue limit in the analyzed sample. The concept of the residue limit in the analyzed sample cannot be emphasized enough [4]. The residue limit in the subsequent product is not necessarily the same as the residue limit in the analyzed sample (although the two can be correlated based on batch size, surface area, and sampling procedure). Some companies have established estringent requirements for their analytical methods because they have established requirements for the methods based on limits in the subsequent product rather than in the analyzed sample. In many cases, the residue limits in the analytical sample are considerably higher (by a factor of as much as 10) than the residue limit in the subsequent product. This is due to the 'concentration" process that results from the nature of the sampling process. In other words, just because the limit in the subsequent product is 5 ppm, one should not despair because one's analytical method only measures down to 10 ppm. If swabbing is done, for example, the residue limit in the analyzed sample may be on the order of 25 to 50 ppm, and a method with an LOQ of 10 pprn would be suitable without further refinement.

SPECIFICITY

In terms of method specificity, there is a natural preference for specific methods. After all, if one has a target residue, the best way to measure that residue is to have an analytical procedure that measures only that species and excludes all potentially interfering species. Specific methods are those methods that target a specific molecule or species and are designed so that possible interferences are eliminated. Specific methods include HPLC, ion chromatography (K), SDSPAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), and atomic absorption (AA). With such methods, it is possible to select, for example, column conditions for HPLC such that the target residue is carefully separated from other interfering species. Such methods sometimes involve some kind of chromatographic separation to isolate the target species to be measured. However, the statement that one should address the specificity of the analytical

method used has sometimes been misinterpreted to mean that only a specific method can be used. It is unclear where this belief came from, but most likely it came from a misapplication of another FDA position on analytical methods. In the early days of cleaning validation, some companies merely analyzed the rinse water as it exited from a cleaned system. If the rinse water met compendia1 specifications (such as USP [U.S. Pharmacopeia] Purified Water specifications), those companies considered the cleaning process successful. The FDA objected to this for several reasons [l ***,3].*** One of the concerns was sampling recovery. Another concern was the fact that the compendia1 specifications may have no relationship to the presence (or absence) of target residue. For example, a residue of a potent active may be present in the rinse water in an unacceptable amount, yet the rinse water may still meet compendial specifications. The FDA indicated that it wanted something that could actually measure the target species. An analytical procedure that can specifically measure the target residue is one way of doing this. However, a second way is to use a nonspecific method, so long as the results of that nonspecific measurement can be *directly related* to the target residue.

NONSPECIFIC METHODS

Nonspecific methods are usually methods that measure a gross property that results from contributions from a variety of chemical species. Examples of nonspecific methods include conductivity and total organic carbon (TOC). Each provides a measure of an overall property but provides no information as to the chemical nature of the source of conductance or organic carbon. When a nonspecific method is used for a target residue, it is necessary to make some assumptions about what that nonspecific property represents. This generally involves expressing the property as if *all* the measured property is due to the target species. How is this done? If one is dealing with a target residue that is an organic active, one way is to measure the TOC of the analytical sample. The TOC value is then expressed as if all the carbon present were due to the target organic residue species. If the amount of the target residue calculated by this method is below the acceptance criterion, then it is scientifically sound to say that the residue is less than the acceptance criterion. For example, TOC could be used, and a sample is found to contain 200 ppb carbon. If the target residue were the active in the drug product that contained 25 percent carbon, then that 200 ppb carbon could be expressed as 800 ppb active. An objection could be made that the organic carbon is not, in fact, due exclusively to the target residue, therefore the method is inappropriate. If the objective were to determine the ***exact*** level of the target residue present, this would be a valid objection. However, the objective is to determine whether the level of the target residue is at or ***below*** the acceptance level criterion. The organic carbon present is probably not due just to the organic active. There may be contributions from the cleaning agent, excipients (for final dosage forms), or processing aids (for bulk manufacture). However, that is beside the point; these facts only strengthen the case for acceptable residue levels of the organic active. As long as the goal is to determine that the ***measured*** amount is below the acceptance level, then good science supports using TOC to reach such a conclusion. Unfortunately, the opposite is not the case; if the TOC measurement indicates that the maximum level of the target residue is ***above*** its acceptance criterion, then one cannot conclusively say that the target residue is above the acceptance criterion established for that target residue. In such a case, one has to either develop a specific method to confirm the exact amount present or use a more robust cleaning procedure so that the target residue, when measured by TOC with all its related assumptions, is clearly below the acceptance criterion. This, of course, should be worked out in the cycle development work before the actual three process qualification (PQ) runs. If TOC were

the only analytical method specified for determining residues, then high TOC values in PQ runs, while not necessarily conclusive evidence of unacceptable residues, would cause the validation protocol to fail.

METHOD VALIDATION

Analytical methods used for measuring residues in cleaning validation protocols should themselves be validated. This validation usually means following standard industry practices for the validation of analytical methods, including evaluation of specificity, linearity, range, precision, accuracy, and LOD/LOQ.

Specificity

Specificity is a measure of the validity of the result based on expected interferences. In other words, one needs to confirm whether or not the method can unequivocally measure the target species in the presence of possible interferences. Methods such as HPLC are generally considered specific. However, they are only specific if possible interferences have been evaluated to see if they change the nature of the assay. For cleaning processes, this means that any HPLC procedure should be evaluated to see whether possible residues from the cleaning agent interfere with the assay. Interferences may include changes in retention time, peak height, or peak shape. If cleaning agents are found to interfere in an HPLC assay, the object should be to modify that assay such that the cleaning agent no longer interferes. Methods such a s TOC or an alkalinity titration are generally considered nonspecific because, in most cases, there is more than one species that can contribute to the measured property. Being nonspecific does not mean that the method is unacceptable. What it means is that there is more risk to the manufacturer in meeting their acceptance criteria. The reason is that a nonspecific method must assume a worst case and calculate a target species as if the measured property was all due to that target species. It is a reasonable expectation that at least part of that measured property is due to the interfering species. However, because one cannot specify that percentage, the worst case must be assumed. With a robust cleaning procedure, such an assumption becomes a reasonable risk. It should be noted that the specificity of a method is not an absolute property but is dependent on possible interferences. It may be the case that what is ordinarily considered a nonspecific method, an alkalinity titration, may be a specific method for potassium hydroxide in the cleaning agent if potassium hydroxide is the *only* source of alkalinity in the cleaning process. In fairness to HPLC methods, it should be noted that if interferences are found, the HPLC method may be modified to account for the interference. With assays such as an alkalinity titration, such modifications are generally not possible.

Range

Range is a series of values of the measured species or property over which the analytical procedure was evaluated. It is only necessary to assure that the procedure is valid over a range of expected values. For example, if the calculated acceptance limit for the analytical sample is X ppm, then one might want to evaluate a range from approximately 0.2X to 1 .OX. On the other hand, if expected results (perhaps based on prequalification studies) are to be in the 0.1X to 0.3X range, then validation of a range of 0.05X to 0.5X may be justified. However, as a practical matter in such circumstances, it makes sense to validate the range up to the 1 .OX acceptance limit to cover the possibility that one data point might be obtained in the 0.5X to 1 .OX range. Such a scenario is generally not worth the risk of trying to shorten the upper end of the validation

range below the acceptance criterion. While it may be interesting to extend the range beyond the acceptance criterion, it is not absolutely necessary. If measured values are obtained larger than 1 .OX, the cleaning validation most likely will be unacceptable.Validating the range beyond 1 .OX will only confirm to what extent those specific values are unacceptable. Determining of the extent of a valid range for the assay is a matter of risk assessment and will depend on the degree of confidence and expected consistency in any prequalification analytical studies.

LOD / LOQ

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

Linearity

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

Accuracy

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

Precision

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

more than one instrument, by more than one operator, or by more than one lab *may not* be needed depending on the specific circumstances related to the individual validation protocol. If the assay is to be used only for validation purposes, less intensive evaluation is needed. If the assay is to be used for ongoing monitoring, then a more elaborate evaluation may be needed.

Keys to Method Validation

It should be noted that in many cases, preferences were given in the discussion of specificity and accuracy. These are not to be considered absolute. In selecting an appropriate analytical method for the validation task, one must balance a series of needs. The key is to be aware of the limitations and risks associated with any analytical method and to take steps to minimize those risks. A robust cleaning procedure is one way to manage the risks related to analytical methods and residue levels. It should also be noted that determination of specificity, range, linearity, LOD/LOQ, precision, and accuracy are ordinarily first done on the analytical method itself, independent of the sampling technique. The sampling technique can affect the analytical method.

TARGET ANALYTES

The analytes targeted for assay will depend on what is targeted in the acceptance criteria. As a general rule, most pharmaceutical manufacturers will have an acceptance criterion for the active ingredient in the equipment cleaned. Therefore, a method to measure that active (either specific or nonspecific) is appropriate. When there is some difficulty in targeting analytes, formulated cleaning agents are often involved. For example, a formulated cleaning agent may contain (in addition to water) a surfactant, an alkalinity source (such as potassium hydroxide), and a chelant. If an acceptance criterion of 10 ppm cleaning agent solids is established, how is that measured? One alternative is to measure each and every species. This makes sense only if an acceptance limit is separately established for each individual component. What is usually done is to target either one component or one property of that cleaning agent formulation 121. For instance, in the example cited above, it may be possible to analyze for the potassium present, and from that potassium value calculate the total amount of the cleaning agent formulation that might be present. If the cleaning formulation solids contained 45 percent potassium, then a measured level of 0.6 ~g potassium would correspond to **1.3** kg of cleaning formulation solids. Such a calculation assumes that the different components of the cleaning formulation are removed from the cleaned equipment at roughly the same rates. While it is possible that there may be differential removal from surfaces, and while it is well known that some surfactants are especially adherent to surfaces, recent work has shown that in a cleaning agent formulation that was freely rinsing, all the components are rinsed at roughly the same proportions within the experimental error of the assay methods used ***[g]*** **.**T he concept of "last to rinse" component is a valuable laboratory tool **[10];** however, as a practical tool for cleaning validation purposes for determining what component to target in a freely rinsing cleaning formulation, it adds little value. Alternatively, a gross property such as TOC or alkalinity can be used to measure residues of cleaning agent formulation. Contributed carbon or alkalinity may be due to a combination of components in the cleaning formulation. However, that gross property may be correlated with cleaning formulation solids. For example, a cleaning formulation may contain 9.7 percent TOC on a solids basis. A measurement of **0.30** ppm TOC would correspond to **3.1** ppm of cleaning agent solids. In this particular case, the issue of nonspecificity comes into play. If there are other possible sources of carbon (actives, excipients), then that **3.1** ppm TOC would actually represent an upper limit for the maximum amount of cleaning formulation solids that might be present. In

either case, whether a specific component or a gross property of the cleaner formulation is targeted, the assumption is made that what is measured is actually representative of the total formulation.

TYPICAL ANALYTICAL PROCEDURES

Below is a short listing of appropriate analytical procedures and their applicability for cleaning validation purposes. This list is not meant to be exhaustive. **High Performance Liquid Chromatography** (**HPLC)** involves injection of the sample into a chromatographic column, separation of the target species from other components in the sample, and then measurement of that target species as it exits the column by ultraviolet (UV) spectroscopy, conductivity, or ELSD (evaporative light-scattering detection). **HPLC** can generally be tweaked such that it is specific for the target species. The equipment is generally available in pharmaceutical facilities.

Total Organic Carbon

TOC involves oxidation of the sample (by any of a variety of techniques) and measurement of the carbon dioxide generated by either infrared spectrometry or conductance. The method is generally considered nonspecific. TOC usually involves an assumption that all of the measured carbon is due to the target species, and the maximum possible level of the target species is calculated based on this assumption. **TOC** is becoming more widely used because it is an acceptable technique to replace for the oxidizable substances test for USP Purified Water and because of the possible degradation of actives due to the cleaning environment. For the latter reason, TOC is used commonly in the biotechnology industry for cleaning validation purposes.

Atomic Absorption

Atomic absorption is a specific method for metal ions. It can be utilized in the determination, for example, of sodium and/or potassium that may be present in cleaning formulations. This is not necessarily a common instrument in pharmaceutical analytical laboratories.

Ion Chromatography

Ion chromatography includes specific methods for both anions and cations in cleaning formulations. It can be used to measure both sodium and potassium as cations, and different methods can be used to separate and measure anions, such a s the anions from acidic detergents (phosphates, citrates, glycolates) or builders (carbonates, gluconates, silicates, EDTA [ethylenediaminetetraacetic acid]). This is not necessarily a common instrument in pharmaceutical analytical laboratories, but it is becoming more widely used.

Ultraviolet Spectroscopy

For certain surfactants that have a chromophore, UV spectroscopy can be an acceptable tool. The instrumentation is readily available in many pharmaceutical analytical laboratories.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is commonly used in the analysis of protein for the determination of actives. However, because proteins are usually degraded by the harsh conditions (temperature and pH) of the cleaning environment, ELISA has limited practical use for cleaning validation studies.

Titrations

Titrations can vary from alkalinity or acidity titrations, which can be used to give upper level estimates of cleaning agents present, to more specific titration procedures to measure components of cleaning agents, such as titrations for chelants in cleaning agents. The laboratory equipment for these procedures is generally readily available.

Conductance

Conductivity measures a nonspecific property of ions in solution. It can be used as an upper limit estimate of the amount of an alkaline or an acid cleaning agent. Dilute solutions exhibit a linear behavior. If not available, the equipment can be purchased relatively inexpensively. Some companies have tried to use pH a s an estimate of residues of either an alkaline or an acidic cleaning agent. This should generally be discouraged. The measurement of pH in unbuffered systems around neutral is unreliable. In addition, the relationship between the level of cleaning agent and the pH is not a linear one. In such situations, it is preferred to use either conductivity or an acidity/alkalinity titration if a simple analytical procedure is desired for cleaning agent determination. pH can be a useful monitoring tool in that a high or low pH can indicate a system out of control. However, it is not a preferred technique for determining actual levels of alkaline or acidic residues.

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