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More on Specificity of Analytical Methods

Judging by the questions I get on the topic, specificity of analytical methods seems to still be a difficult topic for many in dealing with selecting an appropriate analytical method for measuring residues in a cleaning validation protocol. This concern with the acceptability of non-specific methods seems to continue despite the fact that the FDA has clearly indicated that TOC is an acceptable analytical technique if it is used and applied correctly (see my August 2003 Cleaning Memo on "Why TOC is Acceptable").

While some champion the use of TOC as much as possible, in my seminars on analytical methods I generally state something to the effect that "manufacturers should prefer a specific method other things being equal." Why should manufacturers prefer a specific method? The answer is simple: A specific method makes it easier (more likely) that the data obtained will give passing results in a cleaning validation protocol. The reason for this is that the specific method tells you exactly how much of the target residue is present, while a non-specific method represents a worst-case because the measured property (TOC, for example) may be due to species other than the target residue.

Here is a simple illustration. Suppose I have a cleaning protocol with a limit (amount per swab) for the active at 21.2 µg. Suppose further that the actual amount of the active present in a swab sample is 7.0 µg. If I used a specific method (such as HPLC) to measure that active, I would measure (corrected by the recovery percentage, of course) 7.0 µg, and I would pass my validation acceptance criterion. However, suppose I use a non-specific method such as TOC to measure that active. In this case, there may be other sources of carbon, such as the excipients and the cleaning agent, that would also contribute to the TOC value. According to the "rules" of using TOC, I must assume all measured carbon is due to the active, even though I know full well that a significant portion of it might be from sources other than the active. If I were to measure TOC at 13.2 µg TOC per swab, and if the %TOC of the active were 48%, then that measured value of 13.2 µg of TOC represents a possibility of 27.5 µg of active being present. If that were the case, I would fail to meet my acceptance criterion of 21.2 µg of active. Again, under the "rules" of using TOC, I cannot try to rationalize that only a small fraction of my TOC is really due to the active. It is in this sense that it is easier to meet acceptance criteria with a specific method if I could equally choose between a specific method and a non-specific method.

However, in many cases *not all things are equal*. There are cases where a non-specific method generally *must* be used. One case is in biotech manufacture where the active is a protein. In such a case, there may be a specific analytical method, such as an ELISA procedure, to measure the active protein at very low levels. However, following cleaning with hot, alkaline solutions, the protein is usually degraded and not detectable by the ELISA procedure for the native protein. In such a case, the specific ELISA technique is not a "clean indicating" method (analogous to a "stability indicating" method for drug product stability studies) in that the specific method may not be able to distinguish between "acceptably clean" surfaces and "unacceptably clean" surfaces. If the active protein is degraded, then both an appropriately clean surface as well as an unrinsed surface would both show an undetectable amount of native protein using an ELISA method. The converse is not necessarily true. If the cleaning is truly bad (such as failure to clean at all), the specific ELISA technique can determine that the equipment is unacceptably clean (because large amounts of native protein may be on the surfaces). [Note that this does not mean that a specific ELISA technique is of no value for cleaning validation; sometimes it is used to supplement TOC at campaign changeover from one active to another.] Because the

specific ELISA technique is not a "clean indicating" method, it is preferable in such a situation to use a non-specific test, such as TOC or total protein. The TOC value or total protein value is converted (as a worst case) to the amount of active protein for comparison to the acceptance criterion for active protein. This issue of degradation of the active by the cleaning process is not limited to biotech manufacture. There may be cases in cleaning of traditional "small molecule" actives where the active is degraded by the cleaning process and a specific method for the undegraded active is not a "clean indicating" method.

While the biotech case is one where use of a non-specific test like TOC is almost mandatory, there are other situations where a non-specific method is utilized because of *convenience*. In some cases, either a specific or a non-specific method could be a "clean indicating" method, but the specific method is chosen because it is simpler to implement (method development for TOC, for example, may be more straightforward). In such a case, the manufacturer is taking a risk because TOC will be a worst-case measurement tool. The cleaning process must be designed such that in all cases of a properly executed cleaning process, the active *as measured by TOC* is below the acceptance criterion for the active.

The purpose of the Cleaning Memo is neither to encourage the use of specific methods nor to encourage the use of non-specific methods. The purpose is to clarify that either (or both) may be appropriate depending on the specifics of the situation.