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Limits Below the LOD in Rinse Solutions – Part I

Okay, this is another squirrelly Cleaning Memo on using scientific principles (and logic) to deal with a situation that otherwise could cause heartburn. The situation is this. I have established limits in my rinse sample based on carryover calculations, and the limit is X ppm. However, my analytical method can only detect down to a level of 3X ppm. Therefore, it appears that I can't really establish, based on a rinse sample, that I am meeting my acceptance criterion. My first attempt is to try to increase the limit by changing the rinse sampling conditions (usually for rinse sampling this means using a lower volume of rinse). If that doesn't work, I then try to get a better or different analytical method. If that doesn't work, am I stuck? Well, perhaps "Yes" and perhaps "No".

One way to deal with this situation involves situations where I am using a series of discrete rinse steps. That is, after the wash cycle with a detergent (for example), I rinse the equipment once with a fixed volume of rinse solution (this may be a fixed time based on a flow rate). I let that solution drain from the equipment, and I perform a second identical rinse. After draining that rinse, I perform a third or fourth rinse (or however many rinse steps are involved in the cleaning process).

Now if you remember what I have written and taught about rinse sampling, there are two types of rinse sampling. One type is a separate rinse after the final process rinse is completed. The second is to sample the final process rinse itself. For simplicity for this month's Cleaning Memo, I am just going to illustrate this with the second case, that of sampling the final process rinse itself. Also, for simplicity, I will give an example where the rinsing process is a "fill and dump" (or "dump and fill") process. Next month, I will elaborate on application of this concept to a CIP process using a series of successive rinses.

Okay, the picture is this. I have a mixing vessel. I clean it by filling it with a detergent solution, and turn the agitator on for a fixed time. I then drain the detergent solution. Once it is drained, I begin the first rinse by filling the vessel with water, turn the agitator on for a fixed time, and then drain that first rinse. I then repeat that rinsing process several times. I then want to measure the residue of the active (for example) in that final process rinse. But, my problem is that the limit is X ppm and my analytical method can only measure down to 3X ppm. So the best I can do (if I measure residues of the active in the final rinse) is to say it is <3X ppm, which is not good enough to say my cleaning process meets the acceptance criterion. (Note that if I measure a detectable amount, that is ≥3X ppm, in the final rinse, I can clearly say my cleaning process does not meet my acceptance criterion.)

So, here's what you can do. Measure the active in the next-to-the-last process rinse. Now the next-to-the-last of anything is the "penultimate", so I could say measure the active in the penultimate rinse; however, that is not a common term in the pharmaceutical industry, so I will continue to refer to it as the "next-to-the-last" rinse. And let's suppose that I have a quantifiable amount in that sample, for example, 12X ppm of active. Now here's the trick. Can I make certain calculations or assumptions regarding the dilution factor that I would get in going from the next-to-the-last rinse to the last rinse? If so, and if that dilution factor is at least about 1:15 (at least for the example I am discussing), I can reliably estimate that in the final rinse the concentration of the active would be no more than 0.8X ppm. This would meet my acceptance criterion of X ppm (Okay, I would also have to throw in the rinse recovery factor, but you get the point).

So now the question is: How can I reliably determine the dilution factor in going from the next-to-the-last rinse to the last rinse? Before we get to that, this type of calculation assumes that the residue being measured is more or less completely dissolved, solubilized or emulsified during the cleaning process, and that what happens in the rinsing process is that the dissolved, solubilized or emulsified active is removed by dilution with the successive rinses. If there is actually active on the surfaces that has not been dissolved, solubilized or emulsified during the cleaning process, then the active in the rinse solvent would be due both to a dilution factor and to additional active that is possibly removed from the surfaces. However, with proper design of the cleaning process, this should not be an issue.

So, how can I determine the dilution factor? Well, one way I have seen it done is to make estimates of how much rinse solvent is left after a given rinse is drained. This is typically based on a worst case estimate of the thickness of a film of water that could be left on various equipment surfaces (vertical walls, horizontal bottoms, etc.) as well as possible hold-up volumes (perhaps due to drains, dead legs, ports, etc.). I can then take that volume of residual rinse solvent and divide it into the amount of rinse solvent used per rinse cycle to determine a dilution factor. Remember, for this example, we are talking about a dump/fill cleaning operation. For example, suppose this is a 1000 L vessel, and that each rinse is 1000 L. I make a calculation to estimate at a worst case to say that there could be as much as 15 L of rinse solvent left after draining. My dilution factor from one rinse to the next is 1000 L divided by 15 L, or 67. Thus, if I measure 12 ppm of active in the "next-to-the-last" rinse, this means that (other things being equal) that I should measure 0.18 ppm in the last rinse if I had an analytical method that measured that low. However, by this method, I have determined that I have met my acceptance criterion for the active in the final rinse.

Now that is one way to determine the dilution factor. This second method (which will be discussed next) is actually the method I prefer. It is based on getting experimental data on the *actual* rinse steps. One way to do this experimentation is to measure a marker in the last rinse and also in the "next-to-the-last" rinse. This marker can't be the target residue (the active, in the example being used), because the reason we are doing this is that my analytical method cannot detect (much less quantify) the active in the last rinse. Therefore, I may pick another marker, such as phosphate ion. I measure the phosphate ion concentration in the next-to-the-last rinse and in the last rinse, and then divide the result in the next-to-the last rinse by the result in the last rinse to obtain a dilution factor. I could also use non-specific methods as a general measure of what is there. For example, I could measure conductivity on the next-to-the-last rinse and in the last rinse. In this situation, I would not divide the two results. Rather, I would correct each result by subtracting out the background conductivity of the water used for rinsing. Then I would use the corrected results to determine the dilution factor by dividing the next-to-the-last rinse corrected result by the last rinse corrected result.

Another option is to not measure the dilution factor by measuring a marker in the next-to-the-last and the last rinses, but rather measure a marker in the next-to-the-last rinse and in the rinse before the next-to-the last rinse (the rinse before the next-to-the-last rinse would technically be the "antepenultimate" rinse, which takes up less space in a written document, but is not common terminology in the pharmaceutical industry). This is based on the assumption that the dilution factor in going from the next-to-the-last rinse to the last rinse is the same as the dilution factor in going from the rinse before the next-to-the-last rinse to the next-to-the-last rinse. That is a reasonable assumption provided the each rinse is exactly the same. The advantage of doing this is that the marker used is the active itself.

For the experimental results, one option would be to have replicate results for the dilution factor. Provided the

results are similar, the lowest calculated factor from the replicate runs should be used for further calculations. Another option would be to perform the dilution factor by measuring a marker in both the antepenultimate rinse and penultimate rinse to determine a dilution factor, and then measuring a marker in both the penultimate rinse and the ultimate (last) rinse to determine a dilution factor (sorry, but the other terms were just too long). That way, the consistency of the results can help confirm assumptions made. A third option is to measure two markers in the two rinses; theoretically the dilution factor results should be the same. Again, this helps confirm the validity of the results obtained.

This may sound like a lot of work. But, remember that we started with the situation where the detection limit of the analytical method was inadequate. Compared to what would be needed to develop a new analytical method, this option may be the better choice.

I should make it clear, however, that my preference is to have an analytical method with a detection limit at, and preferably below, my limit in the analytical sample. But, the method described in this Cleaning Memo provides a way to deal with situations where that analytical method is not available.

The purpose of the Cleaning Memo is not to advocate for the use of this method for measuring residues in cleaning validation protocols. The objective is to present it as an alternative, and to present considerations in its possible use.