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Dealing with Unknown Peaks

A common question in training seminars is "How do I deal with unknown peaks in HPLC analysis?" My first answer is a facetious one: "Try TOC; there are no unknown peaks in TOC!" However, TOC is not without its problems. To directly answer the question about unknown peaks in HPLC analysis, the first attempt should be to clarify the question. By unknown peaks, we are talking about unknown peaks in the HPLC samples taken in the cleaning validation protocol itself.

The first attempt should be a preventive one. That is accomplished by trying to identify in the HPLC analytical development all possible "unknown" substances. If the HPLC procedure was developed for the active itself, then those other possible peaks that might show up in the HPLC chromatogram include: (1) excipients for finished drugs, (2) process aids, starting materials, or partial reactants for API manufacture, (3) cleaning agents, (4) cleaning process by-products, and (5) sampling materials (swabs, sampling solvents, sampling vials/vessels). For example, take your HPLC method with a known amount of active and spike it with the cleaning agent (perhaps at the cleaning agent residue limit), and perform the HPLC analysis. The cleaning agent may either complex with the active (and perhaps shift the retention time) or it may elute at its own retention time. (With the former, the method needs to be modified to account for the interference. With the latter, a source of a possible unknown peak has been identified.) However, one should go one step further with the cleaning agent and not just mix the active and cleaning agent for analysis, but rather simulate the cleaning process by perhaps heating the cleaning solution and active together. Following quenching of temperature and neutralizing the pH (and diluting to a level around the residue limit of the active), an HPLC analysis should be performed to identify possible peaks which may appear in the chromatogram. These types of tests are the minimum that should be done to identify the source of peaks that may appear in a protocol analysis. By identifying the source of the peaks, the next question that might arise in protocol execution is "Is the peak height or area acceptable?" This requires more judgment, but at least a partial answer can be developed if, based on the discussion in the fourth paragraph, a percentage limit is established for extraneous peaks in the protocol chromatogram. So we'll leave that question until later.

A second way to deal with unknown peaks is to begin your investigation after you have performed the protocol analysis and have identified "truly" unknown peaks (that is, peaks that can't be accounted for by previous studies). In this case, the investigation is expanded. For example, a standard may be spiked with actives (or products) from the previous two or three manufactured products (that is, two or three products before the cleaned product). While these spiking studies should generally be done individually, it should be realized that combinations of previous residues might cause a unique peak. Other things that can be evaluated include various greases/lubricants that might be used on the equipment, as well as materials from any recent interventions, such as a passivation process. Of course, if things mentioned in the previous paragraph were not evaluated before, they should be considered now.

A key question is whether the sources of all the peaks have to be identified. It would be nice to identify all the sources/causes of "unknown" peaks. However, based on past industry experience, this is generally not the case. One possible solution to dealing with these truly unknown peaks is to specify a maximum peak height or peak area that is allowable. For example, it might be appropriate to allow an unknown peak provided it is no more than 5-10% of the height or area of the target residue (the active, for example) at it residue limit. In this

case, it is expected that an investigation has been done to identify the unknown peak, and that it is still remains "unknown". Some companies will then have an additional stipulation that the sum of all peak heights or areas of unknown peaks be no more than 20-40% of the height or area of the target residue at its limit. Of course, the key unknown in this (besides the source of the peak) is the relative UV absorbance of the unknown compound to the target residue. Unfortunately, if the source of the peak is unknown, this amount cannot be determined unless a switch is made to something like an evaporative light scattering detector (ELSD), which can more appropriately quantify the amount of the unknown peak.

If this same issue is applied to extraneous, but known peaks, it is possible to quantify the amount presents by using standards of those "unknown" peaks. If that analysis is done, then the next issue is to determine whether the amount measured is acceptable. If the unknown peak is identified as the cleaning agent, then it may be simply enough to say that the cleaning agent is being measured by a different method, and if found to be acceptable, the cleaning agent in the HPLC chromatogram is not relevant. If the unknown peak is identified as the active of a product that was made several batches ago, then an acceptable limit for that active may be readily calculated. In that situation, it may be also appropriate to modify the cleaning process to be more rigorous to prevent measurable carryover from one batch to a product which is several batches later.

It should be realized that what is appropriately done will depend on the specifics of the situation. However, the best way to deal with unknown peaks is (1) to have a plan in advance to deal with the possibility, and (2) to try to identify in advance all possible "unknown" peaks that might appear in any given HPLC chromatogram.

The issues discussed above also may not be all the relevant issues. For example, some companies might decide to employ mass spec to help identify unknown peaks. The point of this Cleaning Memo is to explore issues that might be addressed with "unknown" peaks in HPLC chromatograms in sample analyses in cleaning validation protocols. The point is not to proscribe nor prescribe certain practices, but rather to define some key issues.