An Executive Summary

EDITORS' SERIES

Controlling Chromatographic Integration to Ensure Data Integrity

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Adequate training and a well-defined set of procedures for dealing with integration is essential element in for a laboratory operating in a regulated environment.

Overview

Regulators are always on the lookout for indications that analysts are testing into compliance. Because manual integration is such a powerful data manipulation tool, it will almost always attract attention. Avoiding problems should start with an understanding of the current regulations.

In recent years, FDA has issued several Warning Letters related to integration issues (see **Figure 1**). In some cases, problems arose from the use of the inhibit integration function without scientific justification. Inhibiting integration is easy to justify at the start of a chromatogram where injection-related baseline perturbations are common. It is much harder, if not impossible, to justify the practice later in the separation.

In another case, data was reprocessed as many as 12 times, raising concerns from FDA. Having to reanalyze numerous times may simply be indicative of a poorly designed method, but certainly raises suspicions that the data was being massaged into compliance.

Another common issue is if the integration of a peak is altered inconsistently across the run or without clear scientific justification. Having an approved procedure for manual integration will not only avoid a lot of these issues, but is also now required by the FDA.

Controlling Chromatographic Integration

Having robust methods and analytical procedures is the best protection against regulatory issues. If manual integration is frequently necessary, that may be an indication of a poor method or insufficiently stringent system-suitability requirements. At all times, whether performed automatically or manually, the integration must be scientifically justifiable. **Figure 2** shows some examples of integration that is likely to raise questions. It is important that standard and sample peaks be treated the same. Applying different criteria to one and not the other will give the impression of "integrating into compliance."

Line 1 in **Figure 1** shows a perfectly justifiable baseline for the first peak, whereas line 2 is an example of peak shaving (i.e., reducing the detected area of the peak). If this were a standard peak, this tactic would enhance the result for the ingredient concentration. Baseline number 3 is an example of peak enhancement in which additional area is added to a peak. Both would be viewed as highly suspicious by a regulatory agency.

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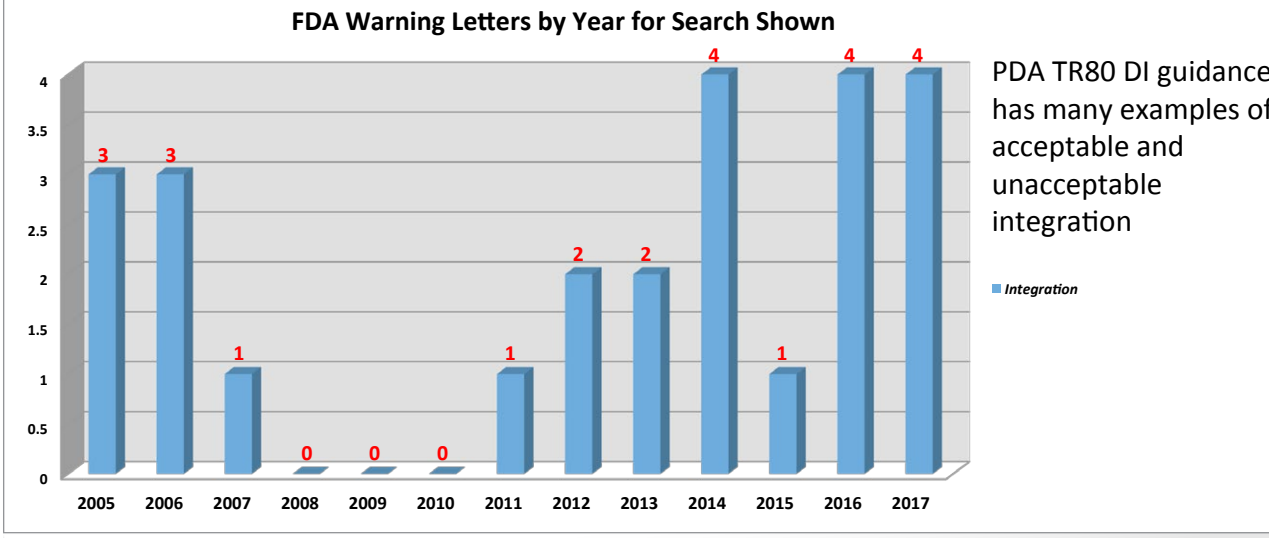
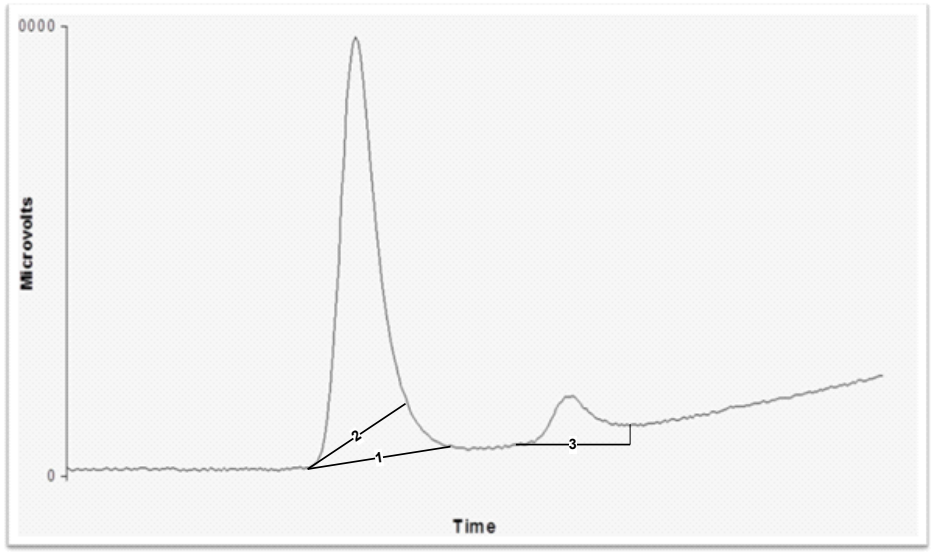
Figure 1: FDA Warning letters citing integration issues.



Graph and data kindly provided by Paul Smith.

Figure 2: Data integrity–shaving and enhancing peaks.



Chromatography is a comparative technique: standards and samples must be integrated the same way

1. Normal integration
2. Peak shaving
3. Peak enhancement

How can we control manual integration?

A laboratory-wide standard operating procedure (SOP) for integration should establish when manual integration is justifiable and how it should be performed in a way that is most sound. All integration attempts must be saved and available for review. If an analyst is found to be repeating an integration multiple times, more training could be needed, the equipment may need servicing, or the method may be flawed. The saving of all attempts should be an automatic component of the data system. It should go without saying that any attempts to bypass the continuous storage of the audit trail will attract regulatory attention.

Adequate training is essential. There is no substitute for a sound understanding of the way that chromatographic baselines behave, and how integration parameters affect the way that the baseline is estimated. Good training will not only minimize risk, but it also saves time and money during internal reviews. The required second-person review of data analysis goes much faster when the procedures have been performed competently and according to standard guidelines.

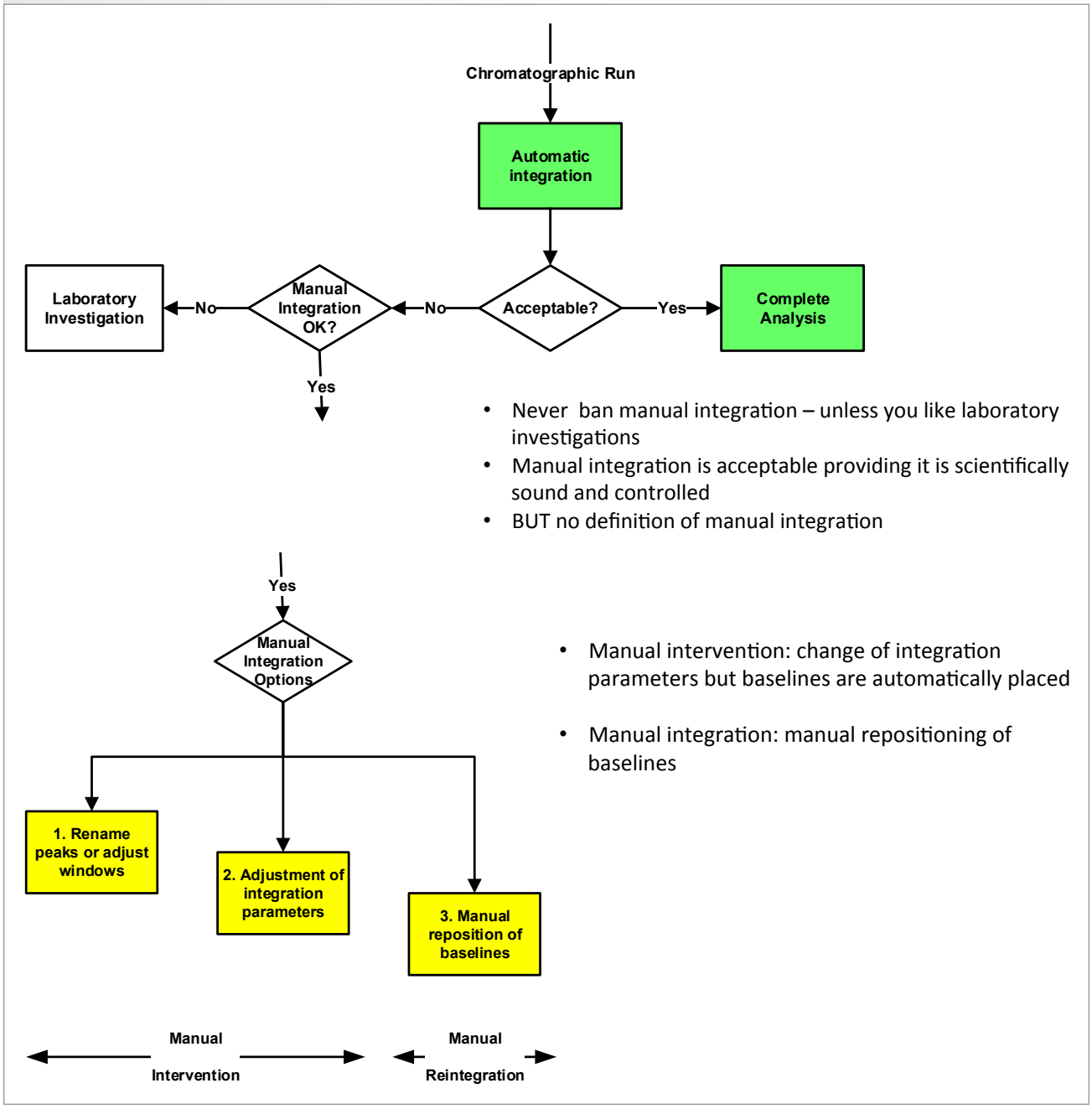
In some cases, it may be best to disallow manual integration altogether. In quality assurance and stability testing of

active pharmaceutical compounds, the peaks are so large and the sample is simple enough that there is little justification for manual integration. In purity determinations, the peak for the active ingredient should probably never be manually integrated, while the much smaller impurities may sometimes require it. In situations where integration is allowed, the number of times it can be performed should be limited. Leaving an audit trail of a large number of reintegrations produces the impression of "playing" with the data to get a desired result.

Integration SOPs

Having a set of SOPs for integration is critical to ensuring proper and defendable processing of data. This should not just include manual integration, but also guidelines on when it should and should not be allowed, as well as guidelines for the development of methods using automatic integration. Ideally, separation and detection conditions should be adjusted so that manual integration is not necessary, but that is not always possible when operating within an already established method.

Figure 3: Manual Integration decision tree.



As a general rule, renaming peaks or adjusting time windows is not a serious change and does not raise concerns. If a peak has clearly slipped outside the integration window, it is perfectly justifiable to move the window. The algorithm for integration has not changed, and therefore the areas have not been changed.

A more drastic intervention is changing the integration parameters. An example where this may be necessary is when the algorithm fails to separate two poorly resolved peaks. Peak areas are likely to be changed in this case. It is important that both standard and sample peaks be subjected to the same change in the parameters.

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By far the most drastic change is the manual repositioning of the baseline. Peak areas will clearly be changed here. This is the procedure with the highest regulatory risk, as the baseline is deliberately moved by the analyst.

Chromatography data systems are intended as a means of converting chromatograms into quantitative results. They should not be expected to make up for poor chromatography. Drifting baselines, poorly resolved peaks, and high noise are all conditions that should be minimized with good system maintenance, robust methods, and stringent system suitability requirements. Suitability tests should not be treated as minimal "rubber stamp" requirements, but as a tool that allows you to avoid time-consuming and scientifically questionable manipulation of poor data.

The method by which chromatograms are evaluated and possibly discarded is important. The greatest danger is to create the appearance that the analyst discarded the run because he or she did not like the result. The SOP should require that the analyst evaluate the quality of the integration before calculation of results (see **Figure 3**). The quality of the chromatography should be evaluated first.

- Is resolution sufficient?
- Is the noise low enough?

Then the quality of the automatic integration should be evaluated.

- Does it adequately track with the baseline?
- Does it distinguish poorly resolved peaks?

If there are issues that justify interventions, it should be performed at this point. Only when the baselines meet approval should the calculations be performed. There should be clear guidelines for how aborted and rejected runs are handled, as well as how to handle extra injections.

Although changing and revalidating methods is a time-consuming and expensive process, the effort can pay for itself if an analytical method requires frequent manual integration. In some cases, specific conditions increase the likelihood that automatic integration will fail. Where justifiable, procedures should be put in place to avoid these conditions such as replacing columns after a specified number of injections. The cost of using more columns is almost always lower than the analyst time associated with manual integration or rejected runs.

The parameters used by the chromatography data system to perform integration should be customized for each method. The default parameters will seldom be sufficient. Ideally, the parameters and the degree to which they can be changed should be codified within the method, as should the conditions that justify manual integration.

In addition to a well-crafted SOP, it is important that the analysts be adequately trained in integration, how the automatic systems operate, and how the data are processed. One good procedure to implement is to have a common set of chromatograms that are used as an integration test. This could include a series of standards or spiked samples with concentrations ranging down toward the limit of detection. Ideally, all analysts should obtain similar areas when manually integrating the same set of chromatograms.

During analytical development, the aim is to reduce manual integration as much as possible. This is the point where the integration parameters are defined for each method before the validation. There should be a set of guidelines for which integration types are acceptable under various conditions. Once validated, the integration parameters should be set by the method. At this point, there should be procedures in place for when the analyst can use manual integration.

Conclusion

Performing manual integration is sometimes necessary, but must be handled judiciously and with a firm understanding of proper theory and practice of integration, as well as the regulatory risks. A written SOP is required, and must clearly define when and to what degree intervention in the automated integration system is allowed, and how it should be performed. Whenever such an intervention takes place, it should be scientifically justifiable. It is safest to make any decisions regarding an intervention, whether that be moving peak windows, altering integration parameters, or performing a manual integration, before performing final calculation.