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Data Integrity in the GxP Chromatography Laboratory

JUNE 2018

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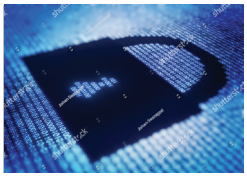

Integration and Interpretation of Data
Mark E. Newton and R.D. McDowall

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INTRODUCTION

Data integrity is a hot topic in the pharmaceutical industry today, and an area of significant concern on the part of regulators. And it's easy to understand why. "Chromatographic analysis can be subverted to manipulate failing results into passing ones and therefore needs to be controlled to ensure the integrity of the results," say Mark E. Newton, the principal at Heartland QA, and R.D. McDowall, the director of RD McDowall Limited. But even if you understand why data integrity is important, you may not understand how to ensure you are following all the rules and best practices.

Reprinted in this ebook on *Data Integrity in the GxP Chromatography Laboratory* are the first three articles in a six-part LCGC series by the same name. This collection, prepared by Newton and McDowall, provides practical recommendations for ensuring data integrity within a regulated good laboratory practice (GLP) or good manufacturing practice (GMP) chromatography laboratory. These principles can be wisely applied by any laboratory that wants to ensure quality work.

In Part One, Newton and McDowall cover best practices for sample preparation, collection, transport, and receipt for chromatographic analysis. The pair explains why investment in automation is critical for avoiding risky manual and error-prone processes. Next, Newton and McDowall explore an essential part of data integrity: how to correctly set up an instrument, run system suitability test samples, and acquire data. The third part of the series, and the last covered in this eBook*, highlights general principles for controlling chromatographic integration and provides a master list of standard operating procedures for such integration.

As Newton and McDowall lay the foundation for achieving data integrity in the laboratory, laboratories will be prepared to comply with GLP, GMP, and GAMP regulations with proper documentation of their analyses.

*Parts IV–VI of this series will be presented in future issues of *LCGC*.

Sampling and Sample Preparation

Mark E. Newton and R.D. McDowall

Data integrity is the hottest topic in the pharmaceutical industry today and many of the issues involved are focused on the chromatography laboratory. Chromatographic analysis can be subverted to manipulate failing results into passing ones and therefore needs to be controlled to ensure the integrity of the results. This article is the first part of a six-part series looking at data integrity from a practical perspective within a regulated good laboratory practice (GLP) or good manufacturing practice (GMP) chromatography laboratory. The principles described here are also applicable to laboratories wanting to ensure quality work. Part I focuses on sample management, transport, and preparation.

This is the first of a six-part series of articles looking at data integrity from a practical perspective within a regulated good laboratory practice (GLP) or good manufacturing practice (GMP) chromatography laboratory. The principles described here are also applicable to laboratories wanting to ensure quality work, such as requirements in International Organization for Standardization (ISO) 17025.

Here, we focus on sample management, transport, and preparation.

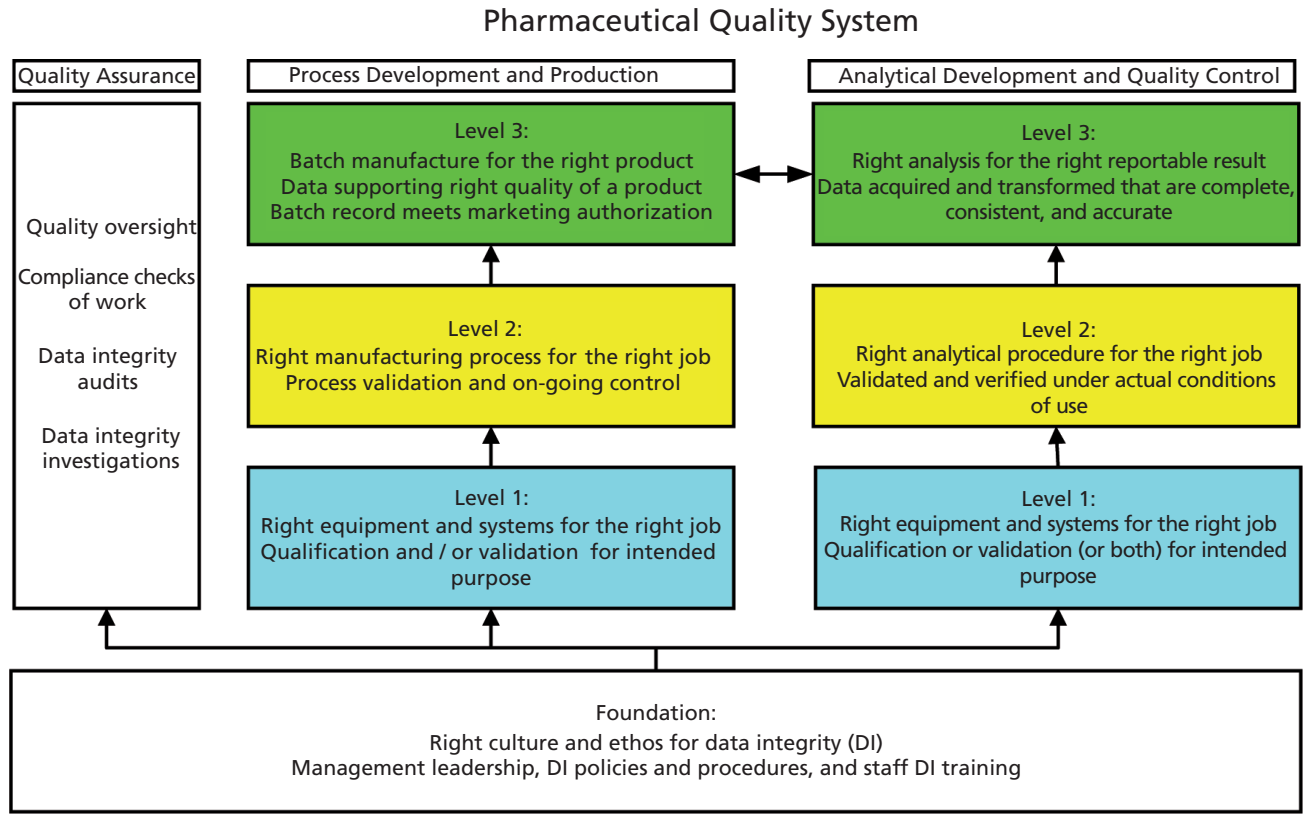
Visualizing the Scope of Data Integrity

To understand the scope of data integrity, a four-layer model has been developed. The full GMP model is shown in **Figure 1** (1) and the analytical portion was discussed in Spectroscopy (2). The four layers are

- Foundation: right corporate culture and ethos for data integrity
- Level 1: right instrument or system for the job
- Level 2: right analytical procedure for the job
- Level 3: right analysis for the right reportable result

In the first five parts of this series we will focus on Level 3, and part VI will look at the foundation layer. Notwithstanding, remember that for the work at Level 3 to ensure data integrity the other three layers must be in place and functioning.

Figure 1: A four-layer data integrity model.



We assume that instruments are qualified and computerized systems and analytical procedures are validated. In addition, readers are aware of the ALCOA (attributable, legible, contemporaneous, original and accurate) principles of documentation that can be found in the appendix of the World Health Organization (WHO) data integrity guidance (3).

The Chromatographic Process

The first five parts of this series will look specifically at the chromatographic analysis as shown in **Figure 2** (4). Here we can see the full scope of an analysis involving chromatography from sampling to generation of the reportable result. At the top of the figure there are seven stages of an analysis with the key items required for ensuring data integrity. There are also

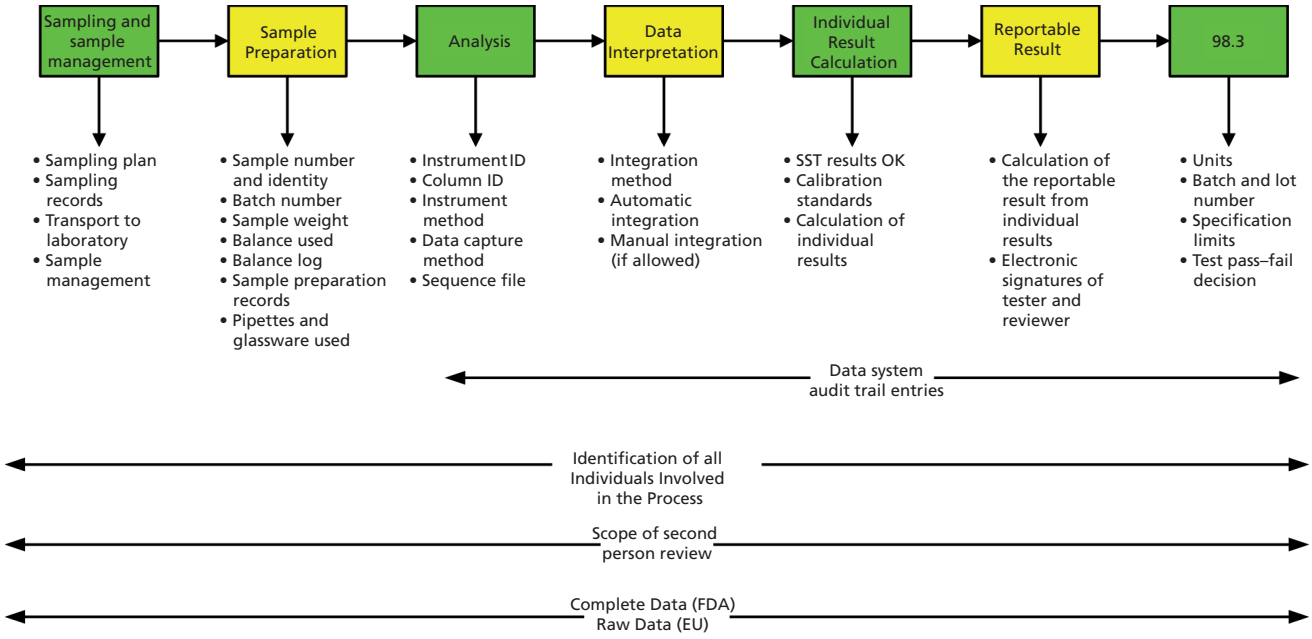
- chromatography data systems with audit trail entries for review,
- scope of the second-person review, and
- what constitutes either complete data and raw data for an analysis.

Data Integrity Articles

The scope of this series of six articles is shown in **Figure 3** and explained in more detail below:

1. Sampling and sample preparation: Two of the most critical areas in analysis that are mostly manual and could be easily manipulated.

Figure 2: The chromatographic process from sampling to reportable result.



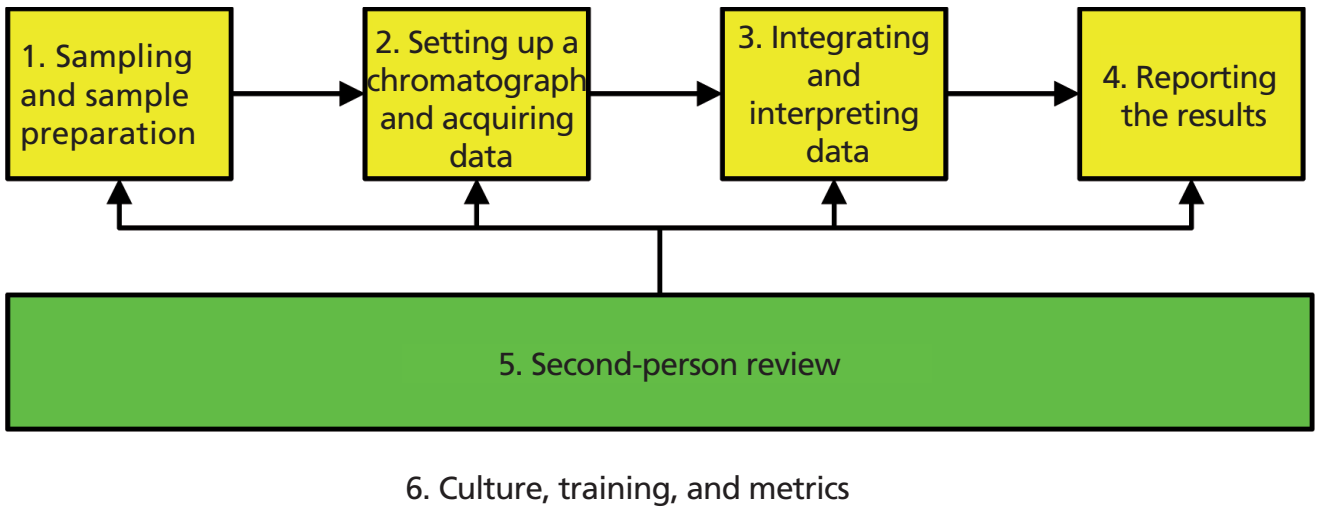
2. Setting up a chromatograph and acquiring data: What should be done to ensure the correct set up of an instrument, how to run system suitability test samples, and the acquisition of data.
3. Integrating and interpreting data: What should be done to control integration and interpretation of the chromatographic runs.
4. Reporting the results: Calculation of the reportable result from an analysis and handling out of specification results and data that have been invalidated in the testing process.
5. Second-person review: Reviewing the records to see that work has been carried out correctly, to ensure that the complete record of testing is present, and to determine if any work is incorrect or potentially falsified.
6. Culture, training, and metrics: Changing behavior in an organization, training for data integrity, and monitoring analytical work in the laboratory.

Data Integrity Issues with Sampling and Sample Preparation

The first two stages of the analytical process (**Figure 2**), sampling and sample preparation, have all the right prerequisites for data integrity issues. Consider the following issues that can be found in most laboratory sampling and sample preparation processes:

- actions are executed by people (sometimes interacting with instruments or systems);

Figure 3: Scope of the articles for data integrity in the GxP chromatography laboratory.



- text, numbers, and labels are involved;
- many steps of the operation are not second-person reviewed at the time of data entry or labeling; and
- errors can lead to incorrect business decisions.

Sampling and sample preparation represents a hidden problem: It is not covered in any data integrity guidance from a regulatory agency. However, if sampling and sample preparation are not controlled, business decisions can be made on inaccurate information.

As we step through this data integrity minefield, consider all the possible answers to the question: What could possibly go wrong?

Current Practice: Sample Collection

Proper sample collection and identification is critical for test result values that accurately characterize the process being tested. Incorrect sample identity can happen in many ways:

- batch printing identification labels from laboratory information management system (LIMS), electronic laboratory notebook (ELN), or lab execution systems offers the opportunity to select the wrong label from the sheet and affix it to the sample;
- incorrectly transcribing the sampling location to the sample container while reading the location from a paper list (or report); and
- wrapping paper labels around samples that are shuffled during transport and incorrectly reapplied to samples.

After sample identity is compromised, data integrity is also lost, and it becomes impossible to reconstruct data with confidence.

In addition to the above errors, consider who will manage them: It is not your senior analyst, who understands the importance of the right sample location and

the right container or transport system to preserve sample integrity. Instead, these errors are managed by the contract person who has a few months on the job, with a minimal amount of training to collect and deliver samples. Faced with the fact that eight sample labels, attached with rubber bands, are now shuffled inside a box, he or she will attempt to correctly affix the labels, rather than report it and risk disciplinary action. As a result of this hidden knowledge, the lab might have a set of atypical results with no assignable cause known for them.

Once sample identity is compromised, a new sample can sometimes be collected, while specific point-in-time samples will simply be lost, as the process has moved past that process point.

So, how can we overcome these potential data integrity issues?

Good Practice: Sample Collection

From a data integrity perspective, a sampling process should be designed to minimize incorrect data entries and to ensure sample identity at all times until sample disposal. It should not permit recording of original data (raw data) on the sample or label, and should prevent the loss of sample information (including identity) from handling and transport.

Best-in-class sample collection processes collect sample data in an electronic system, which allows users to access the system to view sample information, typically with mobile devices. Another advantage is that sample labels include barcoded (or radio-frequency identification [RFID]) number or character strings that can be affixed to containers at any time, because they have no meaning until associated with a specific sample.

This approach allows for a wide range of possible label materials and sampling data to be collected immediately in electronic form using noncontact readers in a mobile computer system. Attribution of action is supplied by the user identity of the person logged into the system account, rather than manual entries. When sampling locations (or other routine sample information) are also barcoded for rapid entry, human data entry is minimized, and the process requires less time. A true win-win situation.

Sample Transport

Some samples require timely transport under specific conditions (for example, temperature or humidity) to ensure accuracy of test results. For such samples, it is imperative to ensure compliant transport to the test facility. Transport can be a weak link in the process, especially when third-parties are involved in transport. Situations where a single temperature or humidity is required for all samples may represent a low risk, but when multiple humidity and temperatures are required for delivery of samples, risks rapidly rise. Remember, the integrity of test results is tied to the correct storage and transport of these samples, which could be in the hands of a person with little training and little understanding of the importance of proper storage and timely transport.

In cases where transport conditions are critical, automated temperature recording with remote data collection is a preferred

solution. At a minimum, calibrated portable data loggers should be transported with the samples to collect data, providing evidence that the samples remained within a controlled environment before receipt. In addition, an investment in transportable data loggers enables the ability to review the real sample conditions afterward. Transport data may hold the key to understanding an unusual laboratory test result from what seemed to be a process in control. Additionally, data logger results can be reviewed and analyzed (such as kinetic mean temperature) to verify the adequacy of the sample, transport, and receipt process. In situations where data loggers are not feasible, sample stability studies can provide evidence that temperature excursions will not significantly impact the reportable test results for specific sample, temperature, or time ranges.

Upon arrival at the testing facility, critical samples should be removed from the transport containers by a laboratory analyst to verify that correct transport containers and conditions were used while delivering the samples. If the delivery person is permitted to remove samples from transport containers and place them in a receipt storage area, information about sample transport conditions is lost.

Sample Receipt

Sample receipt begins when delivery is made to the testing facility. The best scenario is when a trained person immediately receives the samples, records them in the laboratory data system or logbook, and places them in correct storage until testing begins. Reality can be far from ideal: Busy labs may permit samples to sit for as many as several hours before processing and storing them.

Once again, manual data entry provides opportunity for errors in sample locations, sample identity, or sampling comments. In addition, there might be original data attached to the samples (such as paper records) that must be either retained, moved into electronic format, or scanned and retained as an image (true copy). It is important to ensure that all paper records, including information on sample labels, is retained.

The best-in-class receipt process is one that does not require the receiving analyst to make any entry from the keyboard: rather, samples are identified with scanned barcode labels or RFID tags, and comments about the sample are entered directly into the laboratory system by the person who observes them. Unfortunately, this robust approach to data is difficult when external parties are involved in sample collection, transport, or receipt.

Current Practice: Sample Preparation

Sample preparation provides many opportunities to compromise the integrity of test results. Acknowledging that every human interaction is a data integrity risk point, and sample preparation for most methods is a series of manual operations, a "perfect storm" for data integrity lapses is created. Analysts can make small changes in execution that can bias results in a desired direction (toward product acceptance), such as

- adding a little extra analyte,
- slightly under or over-filling a

volumetric flask of the reference standard, and
- recording a slightly different weight for the sample or reference standard.

These are but a few possible risks when preparing samples or standards for assay. These are risks to data integrity over and above those already present because of human errors in weighing, transfer, and manual data recording.

Fortunately, some sample preparation actions can be later verified: For instance, an analyst can view the volumetric flask to verify that 1 mL was removed for a dilution or instrument injection. Weights can be printed to a report and retained for later verification. On the other hand, a weighed powder, dissolved in a liquid, cannot be verified with any certainty unless the operation is observed contemporaneously.

To be trustworthy, manual sample preparation needs a second set of eyes viewing it as it is performed, but this approach is normally seen as economically unfeasible. Therefore, we are then forced to either rely on the quality culture (and lack of incentives or fear) to trust analysts to act responsibly on the behalf of patients, or develop automated (or semiautomated) sample preparation methods that remove the human from the sample preparation process.

The human option—trust your people—has been the only option for laboratories in the past. The automated option was limited and expensive. It was seldom interfaced to other systems for data sharing, and it lacked basic security and integrity capabilities such as individual user accounts. Consequently, beyond high sample volume throughput, there was little else to entice laboratories to move toward automated sample preparation.

The end product is an analyte preparation that is ready to be placed into an autosampler rack or directly injected into the chromatography system. Preserving the identity of the analyte preparation before and after injection is critical. Relying on sample position alone is a risky proposition, because a dropped autosampler can destroy all positions, and errors happen when creating a manual preparation list to correlate sample position with sample identity. As before, the recommended solution is barcoding. Some vendors have begun to offer autosamplers that read barcodes as samples are injected, to ensure the identity of injections sequences. Like sample labels above, preparation containers can have unique number or text sequences that are assigned to a preparation as the container is selected for use. This unique identifier can be recorded (via reader) in the lab records for later review and troubleshooting (if needed). If laboratory records are paper, small labels printed in duplicate can be affixed to the sample preparation container and the other affixed to the paper record. This step creates a means to preserve preparation identity while testing, and for post-assay investigations, if required.

Automate the Sample Preparation Process

The technology option has arrived. Robotics have put automated sample preparation in range for most analytical methods. From a data integrity perspective, it is a win. Au-

tomated preparation removes the human from dilutions and extractions, resulting in greater consistency. It provides accurate and trusted timestamps for all actions—which permit troubleshooting, should the need appear, and prevents ergonomic injuries. In addition, automated preparation generates and records data without human interference. In other words, automation now provides better data integrity in addition to ergonomic and productivity benefits.

Taken together, sampling presents a significant part of the testing process where accuracy and integrity can be lost, often without the ability to reconstruct failing steps. It is an area of regulated operations that begs for better practices and the use of technology to reduce risks in delivering a statistically relevant sample to the instrument for analysis.

Is Management the Problem?

In many organizations, management wants data integrity issues to be remediated quickly and at the lowest cost. Sampling and transport is viewed as a cost to be reduced, with a minimum of attention. This bias has resulted in little attention to the risks (and mitigations) involved in sample management and sample preparation. The problem is that unless there is investment in automation, companies will still be left with high risk manual and error prone sampling and sample preparation processes requiring more effort to review the records and exposure to regulatory scrutiny.

Summary

In the first part of this six-part series we looked at the data integrity issues associated with sample collection, transport, and preparation for chromatographic analysis. In general, these processes are manual and paper based and not covered by any data integrity guidance documents. They are data integrity black holes.

So far, we have traveled in our data integrity journey from the sample to the vial ready for injection into the chromatograph. In part II of this series we will look at the data integrity issues associated with setting up the chromatograph and acquiring the data.

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Address the Paradigm Shift in Regulatory Inspections

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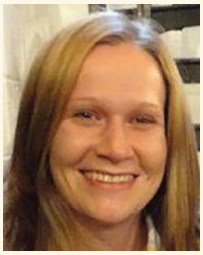
- How FDA inspections have changed
- FDA's goals during an inspection
- The potential systems that may be subject to inspection
- Suggested mechanisms to ensure data integrity in analytical labs
- What type of documented evidence is required to prove that software application systems are validated

Who Should Attend

- Lab managers
- Chemists
- Scientists
- Technical specialists working in industries subject to FDA audits

Presenters

Humera Khaja
Software Compliance Program Manager
Informatics Division,
Agilent Technologies



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Setting up a Chromatograph and Acquiring Data

Mark E. Newton and R.D. McDowall

Correct, complete, and accurate data acquisition is a key component of data integrity. Here we look at how this component should be applied to a chromatograph and a chromatography data system (CDS). In this article, we discuss how to ensure the correct setup of an instrument, run system suitability test samples, and acquire data.

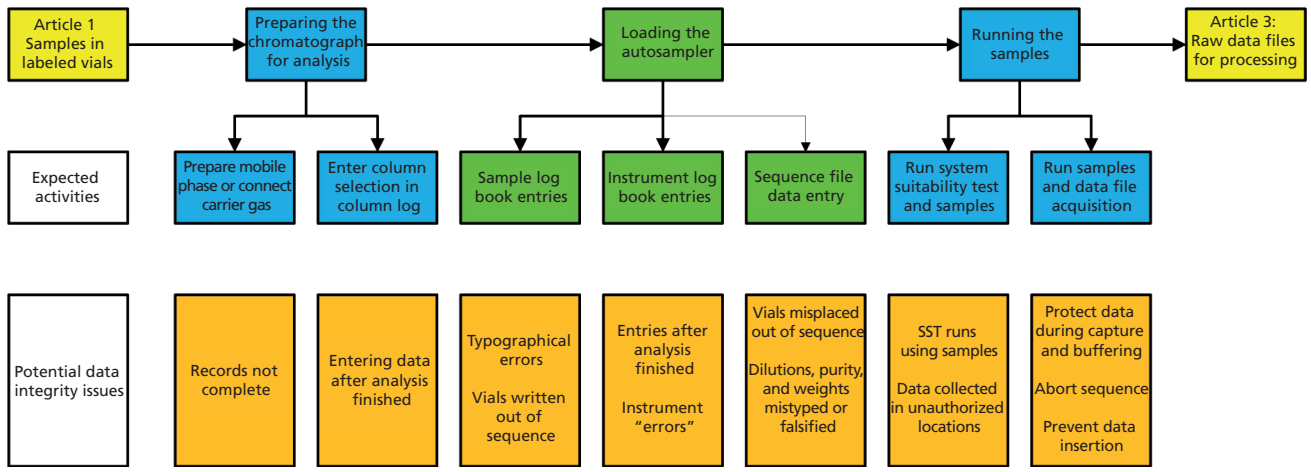
In the first article in this series (1), we covered the sampling and sample preparation phases of analysis and the vials ready for analysis. In this article, we look at what should be done to ensure the correct set up of an instrument, run system suitability test samples, and acquire data. The assumptions we have made are that the chromatograph is qualified and the chromatography data system (CDS) is configured for protecting electronic records, validated, and ideally uses electronic signatures. There should not be any shared user identities so actions in the CDS can be attributed to an individual. For further reading there is an earlier series about the ideal CDS for a regulated laboratory (2–5) and a recent book on the validation of CDS (6). We will not be discussing the instrument logbook because that was the subject of a recent publication (7).

Figure 1 shows what is discussed in this article, and that is expanded in **Table I.**

Managing Factors, Weights, and Other Assay Values in Calculations

Chromatography assays often require the input of additional data values that are combined with the chromatographic value to generate data values that are compared against specifications. For example, the estimated potency, based on the chromatography injection, is combined with the sample weight and moisture results to determine the anhydrous potency, which is the potency value that determines the fitness of the product for release (specification test). Some biologics may have

Figure 1: Setting up the chromatograph and acquiring data.



potency or conversion factors that are applied. Whatever the specifics, chromatography methods require the input of other (external) values to generate results that will be compared to specifications.

These additional values are critical, and directly impact the accuracy of the calculated result; any error in a value will create a corresponding error in the calculated value in every occurrence. Therefore, they must be right. So, how do we get them? Start in a laboratory information management system (LIMS) (or the analytical method) to look up the correct value, then manually enter the value into the electronic method. Some laboratories have developed automated tools to transfer these values from an electronic laboratory notebook (ELN) or laboratory execution system (LES). Automated transfer of these external values can eliminate human transcription errors, but it poses integrity risks of its own if not properly configured and managed:

- Data values manually entered into an ELN or LES, then electronically transferred into a chromatography method, have the same human error risks as data values entered directly into the chromatography system, unless the ELN or LES has some validity checks or other risk-reduction actions embedded in them at the time of data entry.
- When interface files are placed in a target directory to be later collected and processed into the chromatography system, the target directory provides a convenient place for someone to insert an altered data value into the chromatography calculations. This type of manipulation can go undetected, especially if done infrequently. There may be no audit trail for actions in the target directory, and when an audit trail is available, it may be seldom reviewed. A manipulated data set might have a sample weight slightly different from the value in the ELN or LES system—a value that will "improve" the calculated

Table 1: Activities for setting up a chromatograph and acquiring data with their potential impact on data integrity

Activity	Considerations for Data Integrity
Preparing the instrument for analysis	• Calibration considerations • Injection sequences and their management • Sample logbook • Preparing mobile phase and carrier • Column selection and management; column logbook
Loading the autosampler	• Wrong sample identifications • Dropped vials • Incorrect injection sequence • Preserving injection sequence and solutions after assay for potential laboratory investigations
Temperature, humidity, light control	• Potential impact on integrity • Data retention or review of these controls as part of the data set
Acquiring the data	• Permitted changes to acquisition and instrument control methods • Data storage location • Naming conventions used • Aborted runs or short run sequences • Backdoor access to laboratory data servers

Table 2: Comparison of CDS versus ELN and LES to store values for calculations

System Dilutions and Purities Entered Into	Strength	Weakness
Chromatography data system	• Factors and raw data from chromatography are in one place. • Can be easier to review data for investigations (such as out of trend [OOT] or out of specification [OOS])	• Factors, weights can be different in chromatography than raw data source—review required to verify. • Transferred data can be manipulated to appear as original.
Electronic laboratory notebook or laboratory execution system	• Robust audit trail records all changes to factors, weights. Better interface for collecting data values from stand-alone instruments (no manual entry required). • Changes to data values more easily detected and reviewed	• Raw chromatography data resides in another system. • Calculations embedded in electronic method rather than chromatography method

value so it meets the specification. Analysts trust the imported data, because it came in through a validated interface. Only by comparing the chromatography data with the original value can this type of manipulation be discovered.

An alternate approach to calculations is available when the laboratory uses an ELN or LES system. Rather than entering or transferring these additional data values into the chromatography system for calculations, do the opposite: Transfer the basic chromatographic data into the ELN or LES, and perform the post-assay calculations there. Which approach is better? It probably depends on the chromatography system and ELN or LES system in question. In general, data integrity would lead you toward calculations in a location where there is a more robust audit trail, where changes in values are most easily detected, and where recalculations are more restrictive and controlled. Some ELN systems do not—or can be poorly configured to not—provide audit trail records on every action. In contrast, many LES systems create audit trails for every action where any value is overwritten. Chromatography systems vary in their audit trails around calculation and factor changes. The final decision should provide the most robust, secured, and detectable environment for entry and management of external factors, weights, and assay results, as shown in **Table II.**

Obfuscation as a Manipulation Tool

One technique for the willful data manipulator is obfuscation—piling on layers of data to make the truth more difficult to know, to the point that the reviewer finally gives up and accepts the value as genuine. With a chromatography system, obfuscation is easily accomplished by sending multiple data files to a single chromatography run, then perhaps a manual override of a value, followed by another round of data transfer. The resulting audit trail is a tangled weave of changes and timestamps that could be understood—but only with hours of tedious digging. Given other constraints, and the desire to go home at some point, reviewers are tempted to accept the value on their screen and move on. And the manipulator wins. Systems with clear audit trails, formatted for easy review, help to streamline reviews and make it more difficult to use obfuscation. Unfortunately, some systems combine audit trails, adding to the review woes. Quality reviewers should look for test patterns where numerous changes are made, and dig into the data to verify the scientific validity of changes, to stem the use of obfuscation.

System Suitability Test Management

Chromatographic methods have some series of injections that verify the suitability of the instrument before reference standard and sample injections are made. Failure of the suitability test is justification for invalidation of all subsequent standard and sample injections in the test run; therefore, data reviewers must look cautiously at the suitability process, especially when the test run is to be declared invalid along with test results, such as the situation where the system suitability sample, standard, and samples were placed in an autosampler and assayed overnight without human intervention. There are two possible issues:

1. the system suitability samples failed acceptance criteria;
2. the test results failed, and now the analyst is seeking to invalidate the run by manipulating the suitability results.

When test results have been generated, a wise reviewer should look at the potential data values for potential failures as part of the system suitability review.

The potential for system suitability abuse begs a question: Will failures result in investigation and corrective measures? The answer to this question should be included in your procedure on chromatography. In addition, your procedure should also describe the conditions when it is acceptable to perform other types of injections not specified in the method (unplanned), such as injections to verify correct system response before starting system suitability, drift injections, or others. Your chromatography procedure should answer the following questions:

- When are extra (unplanned) injections acceptable?
- If they fail to meet expectations, will they result in repeats or investigations?
- How many repeats are acceptable? When do they stop?
- How will the unplanned injections be reviewed to determine scientific validity?

In a regulated environment, where equipment is qualified and software and analytical methods are validated and users are trained, every injection must be part of the good manufacturing practice (GMP) record.

Incomplete (Aborted) Runs

While collecting data, it may be necessary to abort a run because of any number of issues that impact the test run. But there is also a dark side: Like system suitability, aborted runs can be used as a means of stopping the chromatography system from generating undesired (out of specification [OOS]) test results. Rather than managing the test result, the run is simply aborted, preventing the creation of a result value.

For this reason, it is necessary to manage aborted runs as unplanned events, and review the system for the number of aborted runs, along with the group, person, method, instrument, and abort reason comment associated with the aborted run. The review should look for trends in aborted runs. In addition, it is important to include aborted runs with the subsequent test record, so the aborted data are reviewed along with the acceptable data before test result release. The management of aborted runs should be included in your chromatography procedure.

Other Unplanned Injections

There are circumstances where it may be justified to perform an additional injection of a solution, such as a standard or suitability solutions. As for other solutions described above, it is important to specify the circumstances that warrant these injections, the number of injections (re-injections) that is acceptable, how the results will be used, and confirming that the injection results will be reviewed with all associated test records, to ensure retention of the complete testing record.

Data Output

After the assay has met suitability criteria and injections have been collected, the raw injection results must be stored for processing and assay calculations. One issue that has found its way into some laboratories is diversion of test data into "alternate" directories. This step is more difficult to carry out with relational database systems, but can be readily done with file-based chromatography systems. For example, test runs with failing injections are placed into a folder named "Test" to make them appear as non-production test results (11). This type of behavior can be detected by creating a report to inventory test runs referenced in reported test results, looking for missing runs in the sequence (each run is given a unique identification by the system). A second detection report can look for the presence of folders or injections with suspicious names like "Test," "Practice," or other terms that merit review.

To ensure data integrity, laboratory personnel must be trained to submit every injection for data review. When data are rejected for use, a reason for that rejection must be documented to defend the data exclusion from the reported values.

Is Management the Problem?

Management sets the tone for the laboratory and enforces the expectations. They steer the culture of the firm. Throughout

the process of instrument setup and data collection, management must set the expectation to follow the chromatography procedure and fully report all values generated—whether used for calculations or not. Management is responsible for ensuring that the system is capable of securing data during the testing process, and is configured to capture actions in audit trails that assist the data reviewer to understand the actual actions that occurred. It is especially important for management to expect unplanned injections to be minimized, and not used as a substitute for investigating a substandard instrument or method to understand failures and prevent their recurrence.

Summary

In the second part of this six-part series, we moved from the manual portion of the analysis to the start of computerization and looked at the data integrity issues associated with preparing the chromatograph for analysis and running the samples.

Part III of this series will focus on what should be done to control integration and interpretation of the chromatographic files.

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Integration and Interpretation of Data

Mark E. Newton and R.D. McDowall

Integration is the heart of the chromatographic process and is subject to regulatory scrutiny. What should be done to control integration and interpretation of the chromatographic runs?

This is the third of six articles on data integrity in a regulated chromatography laboratory. The first article discussed sampling and sample preparation (1), and the second looked at preparing the instrument for analysis and acquiring data (2). Now we focus our attention on the integration of the chromatographic data files that we acquired in the last article. **Figure 1** shows what will be covered in this article.

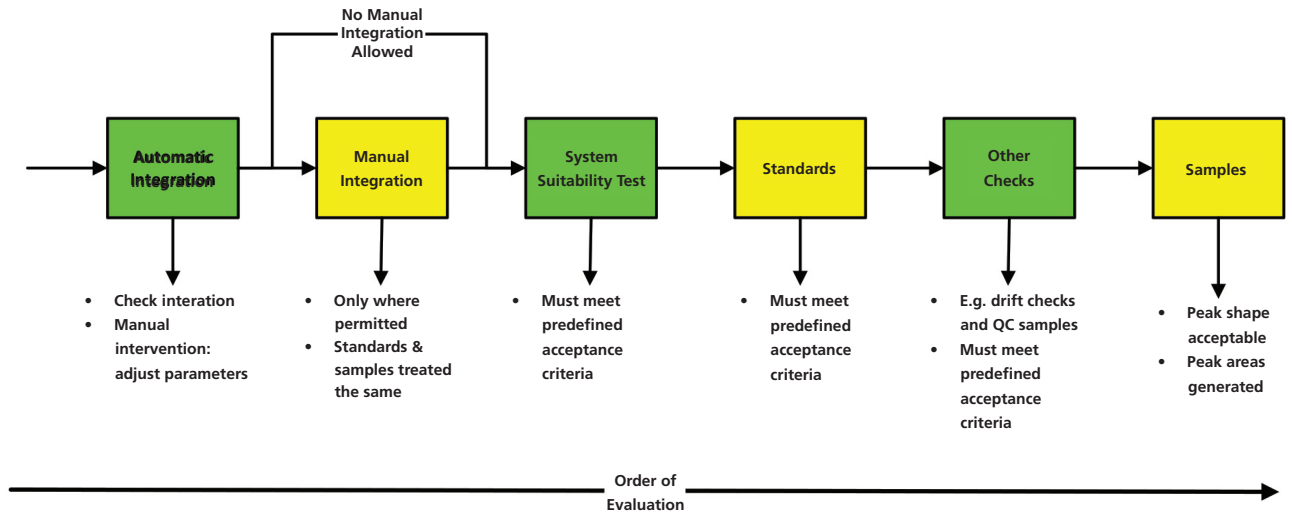
Controlling Integration

Control of chromatographic integration is a key regulatory requirement as discussed in a recent book on the validation of chromatography data systems (3). Lack of integration control has resulted in several U.S. Food and Drug Administration (FDA) warning letters and 483 observations, such as one describing what FDA investigators found at Leiner Health Products (4):

> "In addition, our investigators documented many instances with extensive manipulation of data with no explanation regarding why the manipulation was conducted. This manipulation would include changing integration parameters or relabelling peaks such that previously resolved peaks would not be integrated and included in the calculation for impurities . . .
>
> "There was no Standard Operation Procedures (SOP) to describe the policy, standard practice, and circumstances under which manual integration would be allowed.
>
> "There was no documentation of a justification for the manual integration."

This is not an isolated case. Divi's Laboratories received a warning letter in 2017 (5), stating "Your firm reintegrated multiple chromatograms to determine

Figure 1: Scope and Order of Chromatographic Integration



[redacted] levels; however, the parameters for the reintegration were not retained."

The problem is that regulatory agencies want to see control of integration, specifically manual integration. However, we also need to acknowledge that chromatographic systems are dynamic by nature, and separations can change during a run. Thus, getting the right overall integration constantly throughout a run can be a balancing act. This situation is especially true with long overall runs comprising many samples and injections, with complex separations or biomolecules, where the peak shape is broad.

General Principles for Controlling Integration

Before going into detail for an SOP for chromatographic integration, we need to understand the general principles for good integration:

- Know how key parameters such as peak width and threshold impact the integration of a chromatogram (3,6).
- Never use a default method. Always develop the integration specifically for an analytical procedure.
- Have a robust and reliable analytical procedure; the sole purpose of a chromatography data system (CDS) is not to compensate for poor chromatography—good integration requires good chromatography.
- All integration, in the first instance, must be performed automatically.
- Chromatography is a comparative technique; therefore, standards and samples must be treated the same throughout the run.
- Given the regulatory concerns, only perform manual integrations under conditions permitted in your firm's chromatography SOP or analytical procedures.

An SOP for integration will cover all the above points. However, there is no definition for *manual integration.* We propose the following definition: manual repositioning of the baselines, as opposed to manual intervention, which refers to changing integration parameters (3).

SOP for Integration of Chromatograms

Having established the fact that a chromatography procedure is a regulatory expectation (7), let us turn our attention to the content of such a procedure. We provide a master list of subjects and behavioral expectations. Depending on your organizational approach, some topics might be moved into the training material rather than the procedure. Nevertheless, the topic should be addressed to ensure consistency is practiced and to avoid the appearance of testing into compliance. We will use the previously discussed terms manual intervention and manual integration to describe nonautomated adjustments made to chromatograms by personnel (3,8).

A Robust Chromatographic Integration SOP

The contents of an SOP on integration should include the following items:

1. Definitions: automatic integration, manual intervention, manual integration, analysis, method, test, sample, inhibit, peak masking, processing method. These are among many terms that must be defined for clarity in interpretation among personnel. This need becomes more critical as the number of users and laboratory sites is increased.

2. To set the foundation of the procedure, a set of fundamental behaviors and expectations must be introduced. Without these, the balance of the procedure is not achievable. These expectations must become part of training and include:

- Autointegration of all injection peaks is the expectation. Automatic integration reduces the data integrity risk to the organization, increases consistency in results, and significantly reduces the time to review chromatograms; therefore, manual interventions or integrations are accepted only after no algorithm can be developed to process chromatograms from this material.
- Disabling any audit trail or adjusting a computer system clock connected with the chromatography system is unacceptable.
- Performing any sequence of steps to avoid leaving an audit trail entry is unacceptable.
- Audit trail reason codes entered by users must provide sufficient detail to permit an inspector to reconstruct the sequence and actions performed by laboratory personnel.
- Postinjection data processing will follow a defined sequence: system suitability and criteria acceptance; reference standards and criteria acceptance; drift checks or other checks and criteria acceptance; and samples

and other solutions. A prescribed order reduces the temptation to reject a chromatograph run because of undesirable sample results.

3. A file and data naming convention should describe the proper label for every type of material that will be injected into a chromatography system: suitability, standards, blanks, samples, control samples, drift checks, and so forth. There should be no place for analysts to use informal terms like test, wash, injection, or other such terms found in laboratories by inspectors (9,10). In a regulated environment every injection has a defined purpose; therefore, every injection must have a standardized name. The naming convention must enable the users to quickly understand its purpose. Conformance to a naming convention makes review of data move more quickly—after a convention is in use, nonstandard names are easily seen in a list of sample identifications.

4. Steps to ensure a complete record for review and release: To avoid testing into compliance, the procedure must require personnel to include all injections made while testing, whether they are used to calculate a reportable value or not. Documentation must provide a discussion of data included—and excluded—and the rationale for excluded values. These possible excluded data include aborted runs, runs that fail to meet suitability or method acceptance criteria, excluded replicate values or reinjections of any solution (standard, sample, control, and so forth). In addition, a complete record requires behaviors that create transparency of actions. For example, injections should be labeled as standards, samples, wash, and so forth, before initiating the run. Peak names should be included in the processing method. These actions create an initial record, so changes made postinjection will create an audit trail entry. Labeling injections and peaks postanalysis provides an opportunity for analysts to mislead reviewers by labeling undesired injections or peaks to hide their true content and intent.

5. A process to manage failed (or aborted) runs: Personnel need to have clear understanding of the conditions that permit a set of injections to be stopped or excluded from further use. This process includes documentation within the system, or in other official documentation that provides justification for the exclusion. There must be a means to list the excluded injections in the final review package, because the excluded data must be reviewed for scientific validity before releasing the reportable results.

6. A process to manage extra injections: In this context, extra injections (as defined in your procedure) are any injections of material for chromatographic analysis that are not explicitly

addressed in the written method. A classic example is the injection of a reference standard solution (often the middle standard in a series) to assess the response of the system before initiation of system suitability injections. The suitability injections are specified in the analytical method—but the injection before suitability is not authorized in the analytical method or any other reference document (for example, USP monograph).

7. A process to manage manual adjustments to chromatographs or calculations: All manual adjustments from integration parameters to manual baselines, renaming of peaks, processing calculations and external factors included in reported results, must be transparent to the reviewer and included as part of the test records. In addition, the procedure should describe a risk-based review of manual adjustments. For example, all manual adjustments might require review by a senior scientist with extensive assay experience—someone who is able to assess the scientific merit of the integrations and modified calculations.

Review of an Analytical Run

With the basics under control as previously described, we turn our attention to the integration and interpretation of an analytical run. Here, all samples have been injected correctly and the chromatography separations appear to be acceptable. Now look at the individual chromatograms to see if the integration is correct:

- Evaluate in sequence: system suitability test injections, standards, controls, samples. If any of these items fail acceptance criteria, stop!
- Do not review anything involving samples until assay criteria are met.
- Looking at sample results before accepting the run could be considered "testing into compliance," as if you wanted to invalidate testing you didn't like (or accept, once you did). Fresenius Kabi Oncology received a warning letter in December 2017 for such behavior (11).

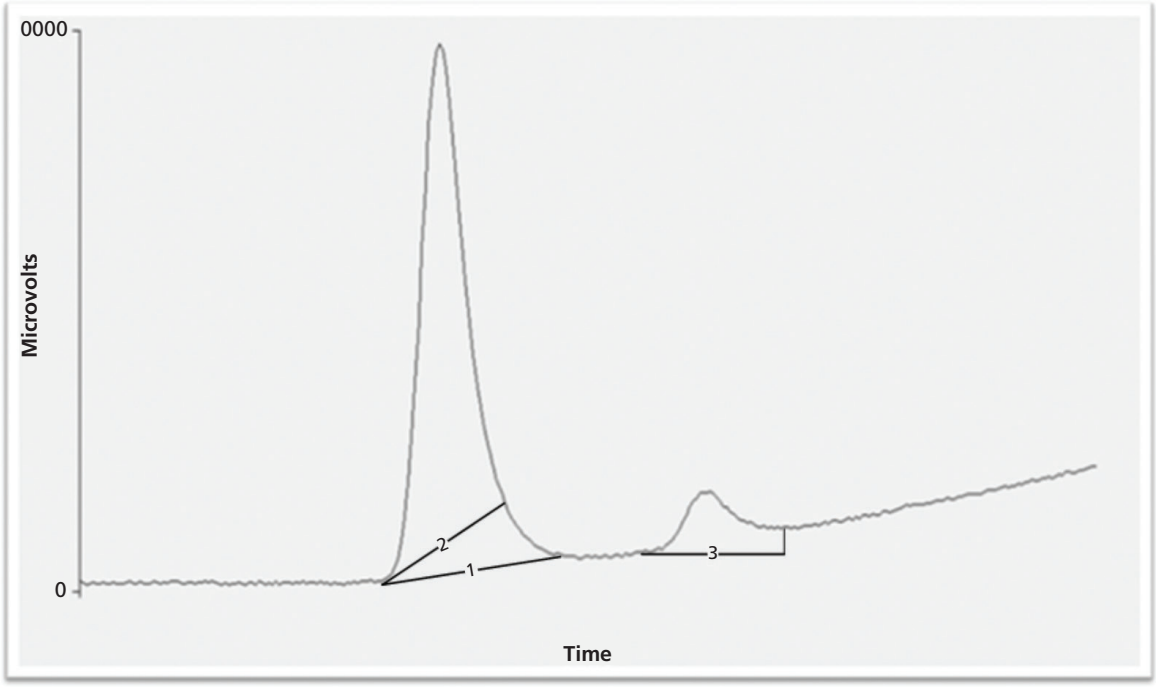
Falsification Practices 1: Peak Shaving and Enhancing

Chromatography is a comparative technique—it compares the response of known standards with unknown samples. It is imperative that standards and samples are processed consistently; otherwise, there will not be a linear relationship between absorption and concentration (Beer-Lambert law). Consistency in peak processing is a strength in automated integration, and one of many reasons for adopting it.

When integrating chromatograms, it is important that chromatographers are aware of bad integration practices used to falsify data (3,8):

- *Skimming* is a technique where the baselines are manually repositioned to reduce the peak area (see line 2 in **Figure 2,** where line 1 is automatic peak placement). If performed to the standards, it can increase the amount of analyte in the standards and vice versa.

Figure 2: Peak Skimming and Enhancement.



- *Enhancing* is the opposite technique, where the peak area is increased for either the standards or the samples to obtain the desired result (see line 3 in Figure 2).

These two practices must be eliminated in all regulated laboratories.

Keeping these out of integrations can be a challenge for biologics that have large numbers of peaks, often broad, because of their natural origins. The first step in controlling these products is admitting their technical challenges—and therefore their risks—and devoting sufficient resources to method development to automate them. It is a tall order to expect consistency from an analyst who spends 4–8 h manually integrating peaks from a single assay, especially when proper assay development could reduce this task to as little as one hour of labor. Dyson (6) recommended that method-related issues be resolved by additional method development rather than chromatographic processing.

Inhibiting Integration

It is difficult to imagine that anyone involved in the manufacture of pharmaceuticals would consider inhibition of peak areas as a good idea, but a warning letter issued to Divi's Laboratories in 2017 lists this approach as a practice in the firm (5).

Failure to ensure that test procedures are scientifically sound and appropriate to ensure that your API conform to established standards of quality and/or purity.

Our investigators observed that the software you use to conduct high performance liquid chromatography (HPLC) analyses of API for unknown impurities is configured to permit extensive use of the "inhibit integration" function without scientific justification.

For example, our investigator reviewed the integration parameters you used for HPLC identification of impurities in release testing for [redacted]. These parameters demonstrated that your software was set to inhibit peak integration at four different time periods throughout the analysis. Similarly, in the impurities release testing you performed for [redacted], your HPLC parameters were set to inhibit integration at four different time periods throughout the analysis.

You have been warned: Inhibiting integration is hiding data from review, just as surely as placing printed batch records in a waste can. There can be circumstances where inhibiting is reasonable, such as with solvent fronts or early baseline instability; these situations will be documented in the validated analytical method. Because of the data integrity risks associated with inhibiting integration, its use should be restricted to validated circumstances within your chromatography SOP (above) and training materials.

Integrating Samples First

One foundation of a robust chromatographic SOP requires that samples be integrated and reviewed last. When analysts are permitted to review sample results first, there is the temptation to find a way to reject (or abandon) the entire run because it is now obvious that an undesired test result will be the outcome. To ensure scientific validity, system suitability should be the first set of injections to be processed and compared against acceptability criteria. A system suitability failure invalidates all subsequent injections (12). Assuming the suitability criteria are met, the reference standard injections should be processed and compared against assay acceptability criteria. Only if all criteria are met should other injections be processed, then samples. Processing samples last ensures that good scientific judgment is driving the process rather than testing samples into compliance.

Linking New Samples to Prior Standard & Suitability Injections

Although it is a common practice to assay additional samples over some time period—say, a work shift—and use the initial set of suitability injections and reference standards as the basis of result calculations, there is a data integrity risk that must be considered along with this practice (in addition to proof that the method remains accurate over the time period): testing into compliance. If an undesired sample result appears, an analyst can reinject the sample preparation again (and again), reference the initial standards to obtain a result, and report the injection that provides the desired test result. This

behavior is detectable with automated searches that look for test runs with few injections—unless you routinely use short runs for samples arriving through the work shift. As a result, the risk–benefit of routinely using short runs should be weighed.

Is Management the Problem?

Outside of examples such as Leiner Health Products (4), where analysts and supervisors were aware of data manipulation practices, most laboratories create data integrity issues through bad practices, combined with a lack of training. Management is responsible for creating a robust chromatographic procedure, and foundational policies that accompany it. These must be incorporated into training that is received by every analyst to assure consistency in practice. Regulators will cite firms failing to provide adequate procedures covering this critical area of the chemistry quality control (QC) laboratory (7).

In addition to procedures and training, management must require metrics that permit both scientific and quality assurance (QA) personnel to monitor the laboratory data for troubling trends:

- The use of short test runs to assay a sample until it meets a specification (that is, testing into compliance)
- The percentage of peaks integrated automatically and manually for each method and laboratory site
- Assays where samples were integrated before standards based on integration date and time stamps

Trending reports such as these permit oversight of routine processes by pointing to trends that merit additional investigation in an efficient manner.

Management taking the short view costs the firm a considerable amount of money each time injections are manually integrated. Time invested to develop automated integration algorithms returns its cost many times over throughout the life of a method and is a solid investment when a long-term view is considered. In addition to time savings, automation provides consistency from analyst to analyst and run to run. This approach is a clear win for the laboratory from bottom to top.

Summary

We have covered integration of chromatograms in this part of data integrity in the regulated chromatography laboratory. To succeed, a robust SOP for chromatographic integration is essential in today's environment. Investment in generating robust analytical procedures where automatic integration is the norm is a big win in time savings and consistency. Also, bad integration practices must be identified and eliminated.

In the next part of this series, we will look at the calculation of reportable results.

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