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System suitability in bioanalytical LC/MS/MS

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Abstract

System suitability is widely recognized as a critical component of bioanalysis. This paper discusses a generic system suitability test that monitors instrument performance throughout a run when used for liquid chromatography tandem mass spectrometry (LC/MS/MS) in bioanalysis. This system suitability process is designed to ensure that the LC/MS/MS system is performing in a manner that leads to the production of accurate and reproducible data that can be submitted with confidence to regulatory agencies. This process contains tests for signal stability, carryover, and instrument response. This approach is integrated throughout an analytical run and has been used in the analysis of over 25,000 batches of clinical samples. Two case studies are presented in which quality control samples and standards meet all acceptance criteria (based on Standard Operating Procedures and the Food and Drug Administration's recommendations for bioanalytical method validation) but failed the proposed system suitability test, and thus were rejected. In these case studies, the concentrations of a significant number of clinical samples (over 35%) were affected, resulting in changes of more than 15% when the samples were reanalyzed. These data indicate that the poor performance of an LC/MS/MS system could adversely affect the calculated concentrations of unknown samples even though the results for quality control samples appear to be acceptable.

Keywords: System suitability; LC/MS/MS; Bioanalysis; Instrument qualification

1. Basic principles of data quality and system suitability

There are four components to ensuring data quality [1]: quality control checks, system suitability tests, analytical methods validation, and analytical instrument qualification (see Fig. 1). Each of these components builds upon the others, enabling an analytical chemist to produce accurate and reproducible data when analyzing unknown samples.

The first of these components is *analytical instrument qualification*. As defined by Bansal et al. [2], analytical instrument qualification is documented evidence that an instrument performs suitably for its intended purpose and that the instrument is properly maintained and calibrated. The process of performing analytical instrument qualification is outlined in Ref. [2] and in the United States Pharmacopeia (USP) Monograph on Analytical Instrument Qualification [3]. Analytical instrument qualification is essential to ensure that the instruments for an analysis are fit for the purpose of the assay and are performing as expected to conduct the analysis.

The second component in obtaining good data quality is *analytical methods validation*. In the field of bioanalysis, analytical methods validation includes all of the procedures that demonstrate that a particular method is reliable and reproducible when used for the measurement of analytes in a biological matrix such as blood, plasma, serum, or urine [1]. This requires that the analysis method be validated in a proper manner to assure that accurate and reproducible results are being obtained. The process for validating an assay has been described for various applications [1,4–8], including bioanalysis [1,4]. In the pharmaceutical industry, the most commonly used reference for method validation in regulated bioanalysis is the U.S. Food and Drug Administration's (FDA) *Guidance for Industry: Bioanalytical Method Validation* [1].

The third component needed for acquiring quality data is *system suitability*. System suitability is widely recognized as a critical component in chemical analysis and is frequently referred to in governmental regulations and guidance policies [1,3,5,7,9,10]. There have been many definitions given for

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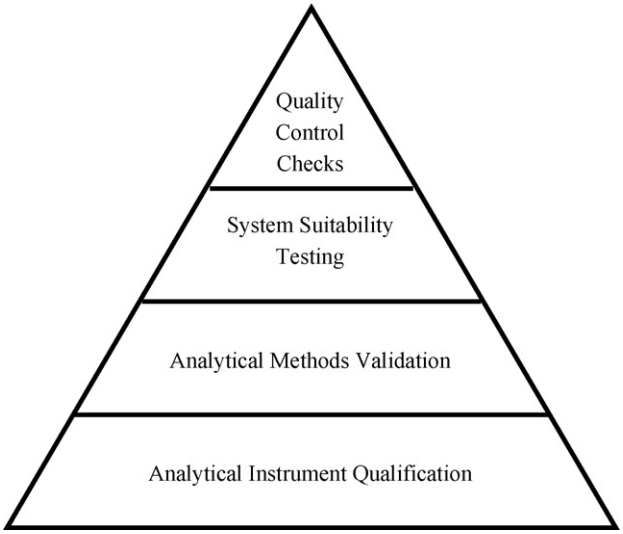

Fig. 1. The components of data quality [3].

system suitability in these references. Hund et al. simply describes a system suitability test as "a test to verify the adequate working of the equipment used for analytical measurements" [11].

The fourth component in the quest for good data quality involves the use of *quality control* (QC) checks or samples. Quality control checks can be performed in various ways, depending on the purpose of the analysis. Typically, an analytical run (also called a "batch" or "batch run" in this paper) will contain standards with known concentrations that are used to calibrate or standardize an instrument's response. It is also common and often required to include quality control samples during an analytical run to monitor a test's performance. The FDA [1] defines quality control samples as spiked samples that are used to monitor the performance of a bioanalytical method and to assess the integrity and validity of the results of unknown samples that are analyzed in an individual batch.

Quality control checks are shown in the top tier of Fig. 1. This is because the performance of standards and quality control checks cannot be relied upon unless the other three components for producing quality data are all performed properly and (in the case of system suitability) monitored for each batch. In fact, it is inappropriate to depend on standards and quality control samples and ignore any of the other three elements in Fig. 1. This will be demonstrated later in this paper, where several case studies will be demonstrate that acceptable standards and quality control samples are not sufficient indicators of system performance. It will also be shown that good performance for standards and quality control samples is not adequate in ensuring that unknown samples have also been measured in a reliable manner.

One challenge in producing quality data is to develop a general way in which system suitability can be determined. Many references have discussed the need for system suitability, but these same sources have been unclear in terms of what procedures should be used to conduct system suitability testing. For instance, the USP [9,10] describes potential testing parameters for system suitability in chromatography [10] and capillary electrophoresis [9] but does not give a defined procedure for implementation in a laboratory. Other sources provide general approaches to determine system suitability in chemical analysis [12,13]; however, only recently has a procedure for system suitability testing in bioanalysis been presented [14]. The remainder of this report will focus on an approach for system suitability testing that can be used for bioanalysis based on LC/MS/MS.

2. Design of a system suitability test

The extent of system suitability testing that is performed by a laboratory can vary greatly depending on the type of analysis that is being examined [2,15,16]. This variety in system suitability tests is probably what has discouraged many authors from describing any given procedure in detail. However, the operation of an efficient, large industrial laboratory (such as those found in bioanalytical contract research) requires a defined and relatively generic procedure for such tests. This is a problem, since system suitability has not previously been described in a sufficiently thorough manner that can be applied to bioanalysis. Moreover, the brief descriptions of system suitability testing that are in previous reports often have significant deficiencies. For example, it is sometimes stated that system suitability is performed prior to the analytical run [1,9], but it will be demonstrated in this report that system suitability should be conducted as a part of the sample batch injection and analysis process. This latter item is particularly critical when using LC/MS/MS instruments, due to the ultra-low limits of detection that are routinely achieved with such devices and the instability of instrument response that is often observed [17–20].

The critical components for the evaluation of system performance in regulated bioanalysis with LC/MS/MS are signal stability, response, and carryover. Each of these items is closely tied to the day-to-day performance of the LC/MS/MS equipment. Fig. 2 shows a system suitability test that has been designed to ensure that these key indicators are being achieved. This includes an evaluation of an instrument's performance and the ability of this device to produce accurate and reproducible data at the beginning, middle, and end of a batch.

The process in Fig. 2 can be broken into three general criteria. First, the analyst must ensure the instrument is performing optimally at the beginning of the batch. Second, the analyst must ensure that the instrument is performing to specifications throughout the analysis of samples, including at the end of the batch. Finally, in the event of instrument failure, there must be a procedure in place to restart a batch to ensure the instrument has maintained or has returned to optimal performance.Prior to placing samples or standards on an LC/MS/MS instrument, it is critical to ensure that the instrument is performing to the system suitability SOP and method specifications for instrument stability, carryover and response (ie. S/N at the LLOQ). For instance, the instrument should be producing a consistent signal. It is not uncommon in LC/MS/MS to have a variable or drifting response [17–20]. To achieve a consistent signal, the analyst will often make many "priming" injections so the instrument will have adequate time to equilibrate [14–16]. Priming injections are simply injections of a system suitability test solution or other sample, with these injections being made prior

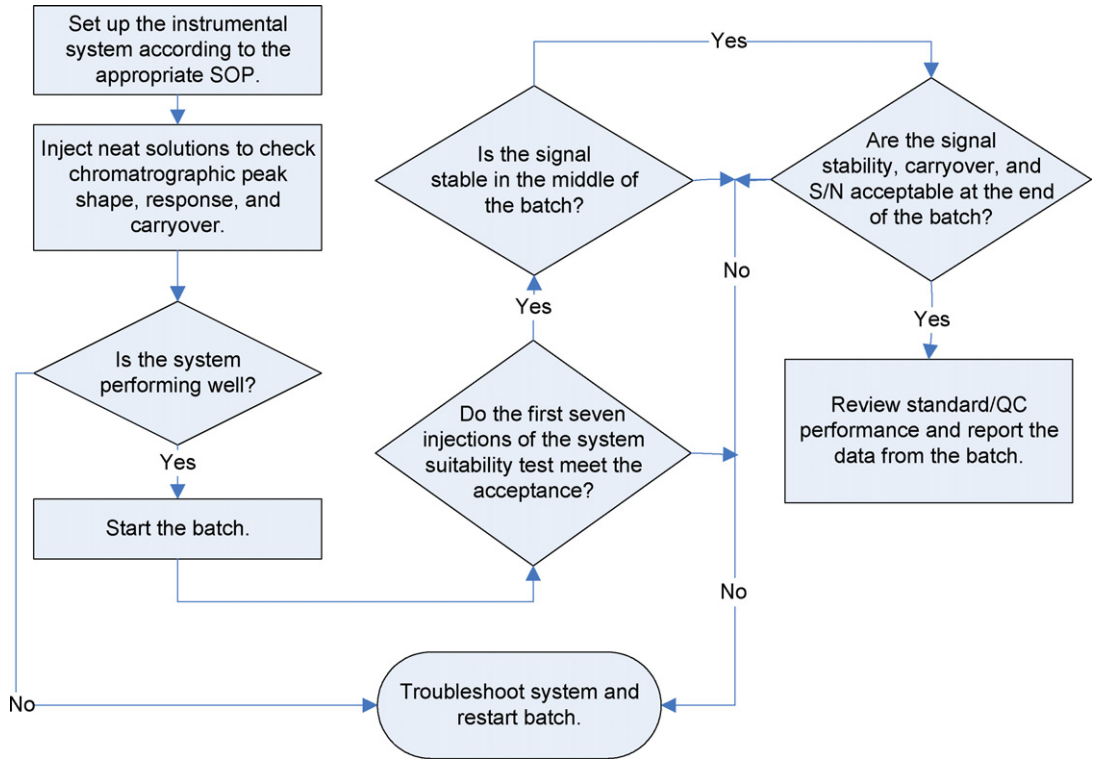

Fig. 2. Flow chart depicting a system suitability test that is used at MDS Pharma Services (Lincoln, NE). *Abbreviations*: QC, quality control; S/N, signal-to-noise ratio; SOP, Standard Operating Procedure.

to the start of the analytical run. Part of these priming injections will also often be used as a check for carryover in the method and instrument response. When an analyst determines that the instrument is meeting the specifications, it is then time to initiate an injection sequence that contains the validation samples (e.g., quality control checks) and unknowns. This is where most system suitability tests end, with instrument performance then being based on the results for standards and quality control checks.

The procedure illustrated in Fig. 2 differs from most traditional approaches for monitoring instrument performance because it performs a system suitability test for LC/MS/MS throughout a batch run. This system suitability test begins with the first samples that are injected in the run's sequence. For example, in Table 1 the first seven injections are all system suitability samples. This is done since it is critical in a regulated laboratory that the official start of an analytical run be documented, rather than simply starting and restarting runs without thorough documentation and justification. These first seven injections are used to evaluate each of the critical parameters that were previously described. When these parameters are evaluated at this point (i.e., after the batch has been started), they are considered part of the raw data that will be available for review and presentation to demonstrate the performance of the assay. During this process, the data from the priming injections are saved but since these samples are part of the instrument set-up, they are not considered when later determining the critical evaluation parameters.

Five of the first seven system suitability injections involve the use of high concentration samples that are used to determine if there is a consistent response from the samples. When using the approach shown in Table 1 for LC/MS/MS, the analyte/internal standard peak area ratios that are obtained for these first five system suitability samples must have a coefficient of variance below 6%. The cutoff of 6% was selected after the review of thousands of LC/MS/MS data sets from many different methods and on over 20 different LC/MS/MS systems in the laboratory at

Table 1
Typical injection sequence used to assay a batch of samples with the system suitability test

Injection no.	Sample type	Injection no.	Sample type
1	High System Suit	42,43	High System Suit
2	High System Suit	44–48	Clinical Samples
3	High System Suit	49	QC Sample
4	High System Suit	50–54	Clinical Samples
5	High System Suit	55	QC Sample
6	Neat Blank	56–60	Clinical Samples
7	Low System Suit	61	QC Sample
8,9	Blank Spiked with IS	62–67	Clinical Samples
10–18	Standard Calibrators	68	QC Sample
19	Blank	69–72	Clinical Samples
20–25	Clinical Samples	73	QC Sample
26	QC Sample	74	Blank
27–33	Clinical Samples	75,76	High System Suit
34	QC Sample	77	Neat Blank
35–40	Clinical Samples	78	Low System Suit
41	QC Sample		

Abbreviations: IS, internal standard; QC, quality control; High System Suit, high concentration system suitability sample; Low System Suit, low concentration system suitability sample; Neat Blank, injection solution (not a biological matrix extract) free of analyte.

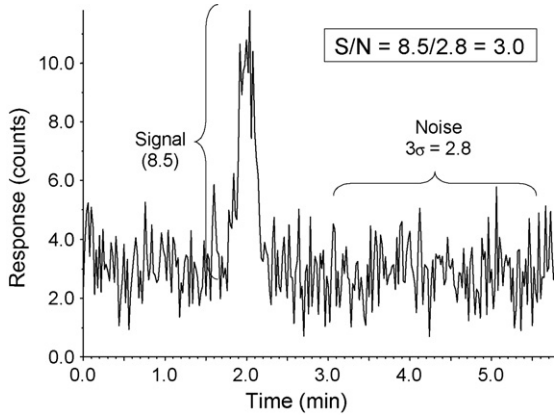

Fig. 3. Determination of the signal-to-noise ratio for a chromatographic peak during the system suitability test. This example is for illustrative purposes only. In practice, the test would be conducted in the same fashion using a validated script provided by Applied Biosystems/MDS Sciex® in which the output appears differently.

MDS Pharma Services (MDSPS) in Lincoln, NE. This cutoff has now been tested for use with the system suitability test in Fig. 2 for over 4 years and has been found to adequately ensure signal stability during this period of time. During this 4-year period, this test was applied to 25,000 batches of samples and over 1,000,000 clinical samples. Several levels of scientific staff and the quality assurance unit of MDSPS critically reviewed each of these data sets for compliance with the "System Suitability Test" Standard Operating Procedure and other applicable Standard Operating Procedures that are directly based on FDA recommendations for bioanalytical method validation [1].

The five high concentration samples used early in the system suitability test are followed by a blank sample extract or a neat solution (i.e., the mobile phase or the injection solution), which are used to evaluate carryover in the method. The permissible level of carryover in this method can be defined in two ways. The first definition uses a default carryover limit of 0.1% (i.e., the integrated area of the peak for an analyte in the neat solution must be less than 0.1% of the integrated area of the analyte's peak in a preceding high concentration sample). The second method used to define the permissible level of carryover is defined by using an FDA recommendation that requires the signal in blanks to be less than one-fifth of the lower limit of quantitation for the analyte [1]. For a standard curve that covers a 200-fold range in analyte levels, this cutoff would again be 0.1%; for an assay with a wider range (i.e., greater than 200-fold), a lower tolerance for carryover (i.e., less than 0.1%) is required.

A low concentration sample is also used for the system suitability test to evaluate the response of the instrument. The signal-to-noise for the response of this low concentration sample must be at least 5:1. For the procedure in Fig. 2, this signal-to-noise ratio is calculated by dividing the peak height for the analyte peak in the chromatogram by three times the standard deviation of a representative noise region in the same chromatogram (e.g., see Fig. 3). This sample and the other first seven samples in the system suitability test are monitored closely, and the run is stopped immediately if any of the test criteria associated with these samples are not met.

Immediately after injection and analysis of the first seven samples in the system suitability test, the standard calibrators, quality control checks, and unknown samples are also injected and analyzed. The system suitability is monitored throughout this run and at the conclusion of the run. This is performed because while it is essential to know that the system is performing well when the portion of the batch containing test samples (e.g., unknowns or validation samples) is started, it is equally important to know that the system performed properly throughout the entire analytical run. The run is monitored by placing pairs of high concentration system suitability samples throughout the batch (i.e., approximately one pair every 40 injections plus one pair at the end of the batch).

The peak area ratio for each pair of high concentration system suitability samples must be within 15% of the mean concentration of the first five high concentration samples. This cutoff of 15% was selected in accordance with the FDA [1] and Standard Operating Procedures at MDSPS, which allow only ±15% variability in the concentrations of the quality control samples versus their nominal concentrations. This cutoff value of 15% deviation has been employed in system suitability tests used during the measurement of over 1,000,000 clinical samples and has been found to be a good index for monitoring instrument stability. During this process, it is important to use high concentration system suitability samples that are identical in composition. This is accomplished by pooling the extracts of a large number of individual samples and dispersing aliquots of this pooled mixture into individual tubes or wells (the latter being used during work with a 96-well plate). Because these system suitability samples should be identical in their content, there is generally no reason for accepting failures outside of the 15% cutoff level. If a high concentration system suitability sample is found to be outside of this range, the batch containing this sample is then rejected.

The only exception to the aforementioned test is if one of the high concentration system suitability samples is found to be a true outlier by Mandel's T_n test [21]. This statistical test is applied to all sets of high concentration system suitability samples for all batches at a 95% confidence interval. This outlier test is used to allow for the possible occurrence of sample pretreatment errors or other causes of experimental variability that are caused by human or pipette malfunction. High concentration system suitability samples that are found to be outliers by the T_n test are rejected individually and are not included in the evaluation of the 15% acceptance criteria for the other high concentration system suitability samples in the batch. However, it is important to note that a maximum of only one high concentration system suitability sample per batch can be rejected as an outlier when applying the T_n test [21].

At the end of the run is a final pair of high concentration system suitability samples, which are followed by a blank sample and a low concentration system suitability sample. These samples are used to ensure that the instrument response has not dropped unacceptably low (i.e., below a signal-to-noise ratio of 5:1) during the run and to help confirm that significant carryover has not begun to occur during the run.

Upon completion of the entire run (i.e., including all of the system suitability samples), a thorough review of the test

Table 2
Acceptable results for quality control samples used in Case Study 1

	Concentration of Quality Control Samples (ng/mL)		
Expected	15	100	900
Measured			
Replicate #1	15.2	93.9	918
Replicate #2	15.5	98.5	925
Replicate #3	12.3[a]	76.6[a]	723[a]
Mean	14.3	89.7	855
%Expected Conc.	95.3	89.7	95
n	3	3	3

[a]The shading indicates quality control samples that do not meet the acceptance criteria defined in MDSPS Standard Operating Procedures.

results for the system suitability samples is performed. If it is found that any of the system suitability tests have failed, the instrument is immediately evaluated and any identified problems are corrected. After these problems have been corrected, the batch is then reinjected. Standard calibrators and quality control samples from the failing run are never evaluated. This approach of not accepting results for batches when the system suitability test fails is consistent with a recommendation from the USP for chromatography [10], which states, "No sample analysis is acceptable unless the requirements of system suitability have been met. Sample analyses obtained while the system fails requirements are unacceptable."

When the system suitability test has passed but standards or quality control samples have failed their acceptance criteria, the batch is not automatically reinjected with the hope that the instrument might perform slightly better. This is because the system suitability test has already indicated that the instrument was indeed functioning up to expectations and, thus, the standards or quality control samples must have failed acceptance due to some other factor (e.g., sample processing).

3. Case studies using system suitability tests

During a system suitability test, it is essential to keep the evaluation of standards and quality control checks separate from evaluation of the system suitability samples. This is because the purpose of these samples is quite different. Standards are utilized to calibrate the response of the method. Quality control checks are used to mimic the treatment of an unknown and to provide an indication as to whether steps such as sample storage, thawing, aliquoting, extraction, analysis, and data integration were performed as expected. The sole purpose of the system suitability test is to ensure that the instrument has performed in a way that will enable this instrument to produce accurate and reproducible data. It is generally perceived that quality control samples can achieve this function of monitoring system performance. However, as will be shown later, quality control samples alone are not adequate for performing this latter function.

One case study is shown in Table 2, which gives the results for a batch of samples that were assayed by LC/MS/MS. This batch

Table 3
System suitability results for the initial and repeated injection of the batch of samples in Case Study 1

	Analyte/IS Area Ratio of System Suitability Samples	
	Initial Injection	Reinjection
Initial Test Samples	1240	1380
	1280	1410
	1260	1380
	1200	1370
	1220	1410
Middle Pair of Test Samples #1	1290	1420
	1310	1430
Middle Pair of Test Samples #2	1320	1370
	1270	1360
Middle Pair of Test Samples #3	1030[a]	1420
	1040[a]	1390
Ending Pair of Test Samples	1140	1430
	1000[a]	1430
Mean	1253	1400
Std. Dev.	54.8	25.8
Coeff. Of Var.	4.4	1.8

[a]The shading indicates system suitability samples that do not meet the acceptance criteria defined in MDSPS Standard Operating Procedures.

of samples was found to meet the typical acceptance criteria for bioanalytical methods as recommended by the FDA [1]. Six of the nine quality control samples in this batch were within 15% of their expected mean concentration, and at least half of the quality control samples at each concentration level were within the 15% acceptance cutoff values. However, this batch was still rejected because it failed to meet the acceptance criteria set for the system suitability test. This is illustrated in Table 3, which shows the system suitability test results. This batch suffered from the phenomenon of instrument drift that is often observed in bioanalytical LC/MS/MS [17,18]. The presence of drift was indicated by the fact that three out of the last four system suitability samples fell outside of the 15% acceptance criteria. Because the system suitability test failed, it was known that the instrument was at fault. After the instrumentation problem had been corrected, the batch was reinjected (as shown in the right-hand column of Table 3), and produced results where all of the system suitability samples now met the acceptance criteria.

The first batch of data in Table 3 would not typically be processed by the approach described in this paper, since this batch had failed to meet the criteria described for the system suitability test. However, in this case study, these data were examined more closely to evaluate the utility of the system suitability test. The results indicated that the performance of the LC/MS/MS method, which had failed to pass the system suitability test in this case, had also affected the accuracy of the final results. A total of 33 of the 112 unknown samples in this batch had concentrations that changed by more than 15% between their first and second injections. In a laboratory in which there is no system suitability test to monitor the performance of an LC/MS/MS instrument (or where system suitability is only performed at the beginning of the batch), this would have resulted in greater than one-third of the results having been reported with significant

Table 4
Acceptable results for quality control samples used in Case Study 2

	Concentration of Quality Control Samples (ng/mL)		
Expected	30	300	750
Measured			
Replicate #1	27.6	265	794
Replicate #2	21.4[a]	272	725
Mean	24.5	269	760
% Expected Conc.	81.7	89.7	101.3
n	2	2	2

[a]The shading indicates quality control samples that do not meet the acceptance criteria defined in MDSPS Standard Operating Procedures.

errors. This could lead to significant problems, particularly in bioequivalence studies, since such errors would lead to a bias in the results between samples from different clinical periods but which were measured in a single batch.

A second case study with a different batch of data provides another illustration in which there was failure in instrument performance (see Table 4). In this example, the performance of the quality control samples would normally be found to be acceptable according to Standard Operating Procedures at MDSPS, since five of six quality control samples were within the 15% acceptance limit. However, as shown in Table 5, the system suitability test indicated that the batch should not have been allowed to continue beyond the first five injections. This occurred since the five initial high concentration samples used during the system suitability test did not meet the acceptance criteria set for signal stability (i.e., the coefficient of variation for the first five samples was 8.0%).

The results for the batch run in Table 5 were compared to those obtained during reinjection to again determine whether the lack of a suitable system test would have significantly affected the accuracy of the results for the unknown samples. It was found in this case that nearly half (16 out of 40) of the unknown samples had measured concentrations that differed by more than 15% between these two batch runs. This again indicated that accepting a run based on the results of only standards and quality control checks was not sufficient in indicating whether proper performance was being obtained by the LC/MS/MS method during an analytical run.

Table 5
System suitability results for initial and repeat injection of the batch of samples in Case Study 2

	Analyte/IS Area Ratio of System Suitability Samples	
	Initial Injection	Reinjection
Initial Test Samples[a]	929	1050
	977	969
	929	1050
	1120	956
	1050	1000
Middle Pair of Test Samples	924	1080
	984	997
Ending Pair of Test Samples	859	922
	932	974
Mean	967	1000
Std. Dev.	77.7	51.3
Coeff. Of Var.	8.0	5.1

[a]The shading indicates system suitability samples that do not meet the acceptance criteria defined in MDSPS Standard Operating Procedures.

4. Alternative approaches for monitoring system performance

Various alternative approaches are frequently considered and used for monitoring system performance. The simplest and most traditional approach for system suitability testing in bioanalysis is to examine the performance of quality control samples that are processed with the unknown samples. This approach has several advantages, such as its simplicity. The use of quality control samples is expected by the FDA [1] and is a component of the Standard Operating Procedures in most bioanalytical laboratories. This means that quality control samples are already in routine use during the processing and analyzing of samples. Unfortunately, the use of quality control checks fails to satisfy the primary requirement for system suitability evaluation since these samples do not separate instrument performance from other analysis components (e.g., sample preparation). Furthermore, as was demonstrated in Section 3 in the case studies, the performance of quality control samples can sometimes be acceptable even when the instrument is not performing properly. As a result, quality control samples by themselves are not sufficient for monitoring system suitability and performance.

When carryover is a concern, it is common for an analyst to deal with this issue through the strategic placement of samples to avoid such effects. As an example, if a particular compound has a carryover of 0.5% and the analytical method covers a 1000-fold range in concentrations (e.g., 1–1000 ng/mL), placing a blank sample following any high concentration samples can be used to minimize or eliminate carryover effects. Because of the 0.5% carryover, a blank injected immediately following a 1000 ng/mL sample would be predicted to have a peak of 5 ng/mL. This would be an unacceptable amount of carryover. The theoretical contribution due to carryover in the sample injected after the blank would be 0.025 ng/mL. This would be an acceptable contribution from carryover. The FDA [1] states that the response for the lower limit of quantitation sample should be at least five times that of the blank. Even if the second sample following the high concentration sample were a blank it would still pass the FDA recommendation because 0.025 ng/mL is far less than one-fifth the lower limit of quantitation of 1 ng/mL. The FDA [3] also states that the accuracy at the lower limit of quantitation should not deviate by more than 20% from the true value. Thus, carryover with a resultant peak that represents a concentration contributing below 0.2 ng/mL would be acceptable for a method that has a 1–1000 ng/mL range.

However, there is a problem with this approach because it assumes carryover will decrease in a linear manner with each following blank injection. In some cases, this may be true or it may be close enough to a linear decrease that this approach will work. However, carryover in LC/MS/MS is often caused by

the adsorption of a chemical to plastic or metal surfaces in the system. Analyte desorbs in subsequent injections in a manner that follows an exponential decay pattern over time. It is not unusual to observe carryover of this type during the development of an assay and occasionally even after an assay has been developed, validated, and used to run many batches. The fact that carryover is often a result of several desorption processes that occur simultaneously in different parts of the system adds further complexity to this problem.

Carryover may also become more extreme throughout a run. This might occur if the tubing of an LC/MS/MS system were not connected perfectly or loosened during analysis, thus exposing the analyte to surfaces it would not otherwise contact. It is also possible for degradation or pitting to occur over time in the surfaces of tubing, needles and columns of an LC/MS/MS system. This latter effect could also change the exposed surface area and materials in the LC/MS/MS system and alter its carryover characteristics with time.

Another problem with the strategic placement of samples and blanks to handle carryover effects is that this approach requires some prior knowledge of the approximate concentrations of the samples. This is not usually possible in settings where it is preferred to conduct blind or partially blind studies, as occurs in a regulated setting using LC/MS/MS for bioanalysis. In addition, it will still be necessary to monitor carryover even when blank samples are used to ensure that carryover is not affecting the results for unknown samples. For these reasons, it is preferable to take measures that help eliminate carryover by treating the sources of this problem rather than simply using blanks to avoid its results and symptoms.

Time spent in identifying and eliminating the source of carryover should always yield better analytical results than simply using blank samples to minimize carryover effects. From a practical standpoint, this approach is more cost-effective for methods that will be used multiple times for large studies or in a large number of small studies. As an example, it has been found at MDSPS that this corrective approach in dealing with carryover has made it possible to develop and validate more than 500 assays of all drug classes over 10 years without the need for placing blanks between samples to eliminate carryover.

The system suitability test in Fig. 2 can also be used to evaluate carryover. In this method, neat blank solutions are placed after high concentration standards. This is useful because it eliminates concerns about matrix interferences being perceived as carryover. This tactic is also acceptable for endogenous compounds that would have a peak in a matrix extract that is far higher than acceptable carryover.

Two other possible approaches for measuring carryover are to use an extracted matrix blank or an extracted lower limit of quantitation sample that immediately follows a high concentration sample instead of the approach recommended in this paper, that is, to use a neat solution. The use of a matrix blank is often the preferred approach because it is possible that extracts from the matrix could have components other than the analyte that adsorb in the LC/MS/MS system. When using a lower limit of quantitation sample, the carryover test would be passed if this sample were within 20% of its expected mean concentration, as recommended by the FDA [1]. While passing this test would give a good indication as to whether the extent of carryover is acceptable, the problem with this approach is that most bioanalytical assays have a lot of variability, especially near the lower limit of quantitation (LLOQ). This makes it necessary to run the LLOQ sample several times within a batch run. Another problem with this approach is that the results of the carryover test would not be known until after all the standards have been injected and analyzed. This is the same reason why the system suitability test described in Fig. 2 recommends the use of a 0.1% carryover or another cutoff that is chosen in a customized fashion based on assay range. The final problem with both of these approaches is encountered when endogenous compounds are being measured. In those cases, the endogenous concentration in the blank matrix is likely to be higher in concentration than the LLOQ.

Instability in analyte response or in the response ratio of the analyte versus internal standard is another common problem in LC/MS/MS. The best approach for dealing with this issue is to utilize a stable isotopically labeled internal standard. This approach is usually sufficient to overcome problems in the stability of the analyte/internal standard response ratio, but there are occasions in which the synthesis of a stable isotopically labeled internal standard is expensive or difficult to perform (e.g., as occurs for glucuronide conjugates or rare drug metabolites).

Another problem with the use of an isotopically labeled internal standard is this compound may not exactly mimic the behavior of the corresponding analyte in an LC/MS/MS method. For instance, this might occur due to different behavior for the analyte and the internal standard during the ionization process. This is a more common problem for deuterated internal standards than those that have stable isotopes of carbon or nitrogen, since deuterium and hydrogen have greater differences in their physical properties than ^{12}C versus ^{13}C or ^{14}N versus ^{15}N. In the case of hydrogen and deuterium, the polarizability of C–H versus C–D bonds is significantly different, which can affect the ionization of chemicals that contain such bonds [22].

Difficulties in obtaining good stability for the analyte/internal standard response ratio can lead to problems with reproducibility and drifting in the value of this ratio. In LC/MS/MS, variability in this response ratio can be 20–50% or more between injections. Drifting in the response ratio occurs when the absolute value of this ratio decreases or increases slowly over the course of an analytical run. Both of these problems tend to be greater in LC/MS/MS than in LC methods using absorbance or fluorescence detection.

To deal with changes in the response ratio, analytical runs are typically designed to have standards at the beginning of the run, with quality control checks, unknown samples, and validation test samples being dispersed throughout the rest of the batch. Many laboratories take one of two approaches to deal with problems with response stability in LC/MS/MS. In the first approach, duplicate sets of standards are used, with one set being placed at the beginning of the run and another set being placed at the end. The second approach is to place the standards randomly throughout the run. Both of these techniques are viable methods for accommodating the response stability problem, but neither gives information on the source of this problem. The

primary advantage of using duplicate sets of standards is that it can accommodate and average out drift that occurs between the two sets of calibrators. However, the use of divergent standards to calibrate unknown samples has been shown to give unreliable results [17]. Randomized standards are also not recommended because response drift is difficult to detect, and using this approach can be prone to carryover effects [23].

5. Conclusions

In summary, a well-defined and scientifically sound system suitability test can be a valuable tool for a regulated bioanalytical laboratory. This test should use predefined acceptance criteria, and the results of each evaluation should be documented according to regulatory standards. An approach for system suitability testing was described in this report that has been used with LC/MS/MS. A variation of this procedure has also been applied for HPLC/UV and ICP/MS methods at MDSPS. Although this procedure has been shown to be successful in a regulated laboratory setting (resulting in higher data quality and better laboratory efficiency), ongoing work is being performed to further characterize and improve upon this procedure. Issues that are still being examined include (1) a search for better statistical tests for evaluating the consistency of the response in an LC/MS/MS method throughout a batch run, (2) an evaluation of the use of a neat solution as a carryover blank when all of the surrounding samples are matrix-based, and (3) methods for removing subjectivity from a signal-to-noise evaluation. However, it is already clear that the system suitability test described in this report can make up a valuable component in promoting data quality in a regulated laboratory setting.

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