The Proper Preparation and Use of Quality Control Samples

Bioanalytical methods used to support clinical and non-clinical studies are dependant upon the use of Quality Control (QC) samples. QC samples are used during method validation to demonstrate many of the required elements such as accuracy, precision and stability. They are also subsequently used during the conduct of the study to provide batch-level quality control. To do this the QC samples must achieve a pre-defined level of accuracy for the associated batch to be considered acceptable. The role that QC samples play is that they represent the matrix of the samples with known amounts of the analyte. The assumption is that if the method performs (either during the validation or routine analysis) in an acceptable manner as measured by the QC samples, then the results obtained for the study samples are valid and can be reported with confidence. This assumption does hold when QC samples are prepared and used in an appropriate manner. There are situations when QC samples fail to provide the expected level of quality control and efforts must be taken to understand these circumstances and avoid them.

Process QC Samples in the Same Manner as Study Samples

One fundamental expectation is that QC samples represent the study samples. When they are processed along with study samples in a batch and perform well, then the analyte concentrations of the associated study samples can be reported with confidence. For this to be true the QC samples must be processed at the same time as the study samples. One action that invalidates the role of QC samples is when the *study samples are processed in a different manner or sequence* than the QC samples. Figure A illustrates a possible scheme followed for the preparation of QC samples used for an ELISA assay. In this case the study samples and the QC samples are diluted up to 90% v/v with non-matrix diluent to minimize non-specific binding. For purposes of convenience and consistency, the QC samples are made up in bulk ahead of time and then frozen, but are diluted prior to freezing. At the time of analysis the samples and the QC samples have had the same actions performed: they have been frozen, thawed, diluted, and analyzed. What is clear from Figure A is that the QC samples and the incurred study samples are not processed at the same time. Because the QC samples were diluted ahead of time, no action actually occurs to the QC samples when the batch is prepared other than thawing and aliquoting. The study samples are thawed , but they are diluted just prior to analysis, so the any error that might be made during the dilution of the study samples will not be reflected in the associated batch QC samples, and thus will not be caught, invalidating the role of the QC samples for batch monitoring purposes. Another problem with this scheme that may not be readily apparent is that QC samples prepared in this manner are not the same matrix as the samples, and so cannot be used to demonstrate long-term frozen or short-term bench top stability during a validation.

Balance Solvent Volumes

Many bioanalytical methods have a sample extraction or preparation step, and rely upon the use of an internal standard for quantitation. When QC samples are processed side-by-side with study samples, they must have the same composition as the study samples in order to draw conclusions about the acceptability of the batch. While both the QC samples and study samples might be expected to be spiked with internal standard (for a typical LC/MS/MS method) it must be recognized that the QC samples are also spiked with the analyte(s) of interest during their initial preparation. Stability and solubility constraints often dictate that analyte and internal standard spiking solutions be prepared in organic solvents. The presence of organic solvents can affect the extraction efficiency and for this reason the overall amount of organic solvent added to all standards, blanks, QCs, and samples must be minimized (<5% v/v) but must also be balanced so that the same amount of solvent is added to every process standard, blank, QC, and sample. This is commonly accomplished by adding additional blank solvent to all of the study samples to ensure they have the same organic solvent content as the QC samples and calibrators. Figure B. illustrates (in a highly simplified fashion) how each of the types of samples (calibrators, blanks, QC, and study samples) have different things added to them, but in the end all have the same amount of organic solvent added and how that amount of organic solvent is kept to the minimum possible. An unintended consequence of failing to match solvent volumes in QC and study samples is that the QC samples will often quantitate well, because the calibrators also have additional solvent, and so behave similarly during sample preparation. So, a batch can appear to pass, based upon the QC sample results, when in reality the study samples behave very differently than expected.

Match Matrix: Liposomes, an Unusual Case

While it is generally well understood that the matrix of the samples must be matched in the preparation of the QC samples there is one common situation where this is not well understood and poorly practiced: liposomes. Liposomes are utilized in many drug product development programs to increase circulation life, reduce unwanted side effects, and target medication to specific sites. Not only must a bioanalytical method be able to differentiate free drug active from encapsulated (intact liposome) drug active, but the QC samples must also contain sufficient liposome material to account for the amounts of liposome likely to be found in study samples. Liposomes are principally composed of phospholipids. Methods designed to dissolve or disrupt liposomes can be defeated if there is more liposome in the sample than anticipated, and even disrupted, the phospholipid material can dramatically affect extraction efficiencies. Animals in GLP studies tend to be dosed at much higher levels (volume of liposome to volume of blood) than humans and so QC samples used to validate these methods and monitor these batches must have higher amounts of liposome material in them to adequately match the study sample matrices.

Figure A.
QC Samples Processed Differently than Study Samples

QC Sample Preparation	Study Sample Preparation
QC Stock Solution	Sample collected
⇓	⇓
QC Sample prepared in 90% diluent	
⇓	⇓
QC Sample (frozen)	Sample (frozen)
⇓	⇓
QC Sample thawed	Sample thawed
⇓	⇓
	Sample prepared in 90% diluent
⇓	⇓
QC Sample aliquoted and analyzed	Sample aliquoted and analyzed

Figure B.
Solvent Balancing in Calibrators, blanks, QCs, and Samples

Item	**Calibrators**	**Blanks**	**QCs**	**Samples**
Blank matrix	0.990 mL	0.990 mL	0.990 mL	0.0 mL
Sample matrix	0.0 mL	0.0 mL	0.0 mL	0.990 mL
Int. Stnd. Soln.	0.005 mL	0.005 mL	0.005 mL	0.005 mL
Cal. Stnd. Soln.	0.005 mL	0.0 mL	0.0 mL	0.0 mL
QC Stnd. Soln.	0.0 mL	0.0 mL	0.005 mL	0.0 mL
Blank Solvent	0.0 mL	0.005 mL	0.0 mL	0.005 mL
Total Volume	**1.000mL**	**1.000mL**	**1.000mL**	**1.000mL**
% Organic Solvent	**1.0%**	**1.0%**	**1.0%**	**1.0%**