



Sensitive bioanalytical method for biologic

We have a protein-based therapeutic in our development pipeline that is expected to metabolize quickly. We require a sensitive method for determining blood levels of the drug. What is your approach?

A close collaboration with the client during the early method development stage, and throughout the development life cycle, is essential for the generation of a successful assay. The key factor responsible for providing a high level of sensitivity is the production or procurement of appropriate critical reagents (high-affinity capture and/or detection antibodies). A high-affinity antibody may lose its ability to provide sensitivity if a high minimally required sample dilution is necessary to avoid selectivity or matrix effect complications. Investment of time and effort to develop specific, selective, and high affinity reagents well in advance of the intended preclinical or clinical study start date will allow for the development of a successful and on-time bioanalytical method.

With candidate-critical reagents in hand, it is now possible to investigate how these can be combined to maximize assay sensitivity in the intended study matrix, culminating in the generation of a prototype assay.

Many protein-based therapeutics have short, circulating half lives, thus requiring, optimized procedures to prevent metabolism during or after sample collection, and these must be established up front using the prototype assay.

An appropriate sample collection procedure can be established by investigating the use of suitable enzyme inhibitors or other stabilizers. Rigorous bench top and extended storage stability testing should be performed to determine whether such stabilizers/inhibitors are to be added to the processed matrix at the time of sample collection, or to the collection tube in advance of the collection time. Such investigations will also ascertain whether or not the appropriate matrix is being analyzed in the assay (whole blood versus plasma/serum).

With the prototype assay in place, and the study matrix established, further refinements to increase the level of sensitivity may be applied, if required. This may include optimization of the matrix, assay buffer conditions, or the platform utilized in the assay. Electrochemiluminescent (ECL), fluorescent, or radioactivity-based approaches may be investigated, as these have often been shown to provide a higher level of sensitivity than conventional colorimetric methods.

Once the intended final assay format has been established, we apply a pre-validation screening protocol, which includes assessments of selectivity in different lots of matrix (spiked and un-spiked); dilution linearity; high-concentration hook-effect (i.e. prozone evaluation); precision and accuracy assessments; as well as preliminary matrix stability evaluations. This evaluation determines whether the method is suitable for proceeding into Good Laboratory Practice (GLP) validation by demonstrating that it yields quantitative data within pre-defined acceptance criteria in line with the relevant guidance documents.