The Development and Validation of an Enzyme Immunoassay for the Determination of Exenatide (Exendin-4) in Human Plasma for Pharmacokinetic Analysis

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PURPOSE:
- Exenatide is a synthetic version of exendin-4, a polypeptide found in the saliva of the gila monster. Exendin-4 displays biological properties similar to glucagon-like peptide 1 (GLP-1). Exenatide has been developed for the treatment of diabetes mellitus type 2.
- Commercial immunoassay kits for exendin-4 were evaluated and matrix effects in different lots of human plasma were observed. A commercial kit was adopted and validated to meet the FDA guidelines for validation of immunochemical methods.

METHOD:
- Aprotinin-treated samples and calibration standards were treated with a protein precipitation reagent and the supernatant evaporated and reconstituted in a buffer solution. Exenatide in the solution was allowed to bind to the binding sites on the exendin-4 antibody prior to aliquoting onto a microtiter plate coated with a secondary binding antibody. Exenatide in the immunoassay competed for binding to the exendin-4 antibody with a fixed amount of labeled exendin-4 added to the plate. The plate was washed to remove any unbound reagents from the plate. An enzyme substrate was added, and allowed to bind to the standards attached to the plate, and unbound material was then washed away. A fluorescent substrate was added to the wells and the signal resulting from the cleavage of the substrate was read on a fluorescence plate reader.

DEVELOPMENT:
- Method development started with comparison testing of exendin-4 assay kits from multiple manufacturers, utilizing different detection technologies (EIA, FIA, RIA). A fluorescence immunoassay (FIA) was eventually selected for further development, based on the sensitivity and reproducibility of the assay. A concentration time was run when back-calculating fortified human plasma samples against calibrators prepared in the kit supplied buffer, this time was resolved by preparing calibrators in human plasma.
- Aprotinin treatment of the samples was included to ensure that exenatide and exogenous peptides did not degrade, and testing spiked samples incubated with and without aprotinin demonstrated that the addition of aprotinin did not have an effect on the quantitation of exenatide. Whole blood samples were fortified with exenatide, incubated at 37°C, then centrifuged and the plasma treated with aprotinin. These samples quantitated similar to whole blood samples that were centrifuged immediately after exenatide fortification and the plasma subsequently treated with aprotinin.

RESULTS:
- During the initial qualification of the immunoassay method, matrix effects in multiple lots of human plasma indicated the need for a fortification step to ensure adequate quantitation of both native samples and samples fortified with exenatide. Since exendin-4 in exenatide is not identical, we were unable to measure intact levels, but rather intact exendin-4. The sample was affecting the results. A protein precipitation step was included, followed by evaporation and reconstitution of the supernate, produced more sample results below the limit of quantitation and accurate quantitation of fortified samples. This was later modified to precipitation with trichloroacetic acid, which gave better recovery of exenatide from the samples and provided more efficient evaporation of the supernate.

CONCLUSIONS:
- The method allows for rapid, sensitive, accurate and reproducible quantitation of exenatide in human plasma samples for pharmacokinetic evaluation.