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Title: Development of new bioanalytical method and optimization of validation procedures

Abstract

A crucial component of the research and development of new medicinal products involves pharmacokinetic studies. These studies aim to determine the concentrations of the active substance and its metabolites in the biological matrix, typically in human plasma, utilizing validated bioanalytical methods. Nevertheless, there remains an unmet need for the development of new bioanalytical methods.The validation of bioanalytical methods adheres to the guidelines set forth by regulatory agencies such as the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA), while documentation is in accordance with the principles of Good Laboratory Practice (GLP). In 2019, the International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) introduced the draft of the ICH M10 guideline, representing an effort to globally standardize the validation of bioanalytical methods—an aspiration held for several years. In light of ongoing work to finalize the ICH M10 guideline, it became evident that optimization of certain aspects of validation tests was necessary. These aspects included the recommended number of quality control (QC) samples in stability tests, the order of sample order within the analytical sequence during matrix effect tests, and the utilization of multiple sources for matrix effect tests. Additionally, a less understood area pertained to the long-term stability of analytes within biological samples.

This study aimed to achieve several objectives: 1) Develop new bioanalytical methods. 2) Optimize validation tests, specifically within the matrix effect test (considering the order in the analytical batch) and the stability test (determining the optimal number of QC samples). 3) Evaluate the long-term stability of analytes, specifically dutasteride and aripiprazole, in human plasma.

Two new methods were successfully developed validated for the determination of 21 antidepressants, as well as for dutasteride (a synthetic analog of testosterone) and its metabolites in human plasma. These methods can be applied in bioequivalence studies. Furthermore, the long-term stability of dutasteride (over a period of 3 years) and aripiprazole (over a period of 7 years) in human plasma was proved.

The results of the optimization study for matrix effect tests highlighted the importance of considering the diverse phospholipid profiles present in lipemic plasma samples, which significantly contribute to the observed matrix effect. A single source of lipemic plasma was deemed insufficient for assessing the reliability of bioanalytical methods. Regarding the stability test, it was examined whether the draft ICH guideline M10's recommendation of a minimum of three QC samples adequately demonstrated analyte stability. The study results indicated that five QC samples represent the optimal number for this test. The findings generated from these comprehensive studies were incorporated into the updated edition of the Standard Operating Procedure for bioanalytical method validation within the Pharmacokinetics Section.

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