DEVELOPMENT OF A HIGH SENSITIVITY UPLC/MS/MS METHOD FOR THE ANALYSIS OF CLOPIDOGREL AND CLOPIDOGREL CARBOXYLIC ACID METABOLITE IN HUMAN KETA PLASMA

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INTRODUCTION

Another essential part of method reliability and robustness is the column. Various columns, such as C18, C8, C4, Zorbax, and Hypercarb, were evaluated in the present study. The results showed that the C8 column provided the best performance for the separation and quantification of clopidogrel and its metabolite. Furthermore, the use of solid-phase µElution SPE as the extraction method allows the sample to be concentrated (without an evaporation step) by a factor of up to 50 µL of water. This yields a 3.5X concentration of the sample prior to the injection.

RESULTS

To assess method carryover for both clopidogrel and its metabolite, an extracted plasma blank was injected immediately following the upper limit of quantitation (ULOQ) standard of 500 pg/mL. The resulting chromatogram showed no detectable response for either clopidogrel or the carboxylic acid metabolite at the appropriate retention times (Figure 4), indicating that no carryover was present in the assay.

In order for a bioanalytical method to be used for pre-clinical or clinical work, it must first go through a rigorous validation process following the guidelines set forth by the FDA. Part of the validation process involves demonstration of accuracy and precision of the assay. For this reason, ANOVA and %CV were used to determine the acceptable range of results. The results showed that all detected samples were within the acceptable range (Figure 5), suggesting that the method is accurate and precise enough for clinical use.

CONCLUSION

• A high sensitivity method for clopidogrel and its inactive carboxylic acid metabolite was developed using solid-phase µElution SPE as the extraction method.

• The assay was linear over the range of 0.0005–500 pg/mL, and showed no detectable carryover for the drug or the metabolite.

• Statistics for 4 replicate samples for each 4 QC concentration showed the intercept and slope values were within the acceptable limits of the FDA guidelines.

• Both analysis of interest were successfully separated from potential co-eluting compounds.