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Development of validated LC-MS methods using surrogate matrices for the quantification of biomarkers of clinically significant drug-drug interactions

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Intro

When developing a quantitative LC-MS/MS method, ideally the standard curve and quality controls should be prepared in a matrix as similar as possible to the study samples to limit the potential for dissimilar matrix components to interfere with accurate quantification. However, if blank matrix is unobtainable, or if the analyte of interest is endogenous, then use of a surrogate matrix may be necessary.

The plasma ratio of caffeine/paraxanthine is used as a reliable indicator of the drug metabolizing enzyme CYP1A2. Similarly, coproporphyrin I/III are used as biomarkers of potential drug-drug interactions mediated by hepatic transport. The widespread use of caffeine makes obtaining drug-free plasma impractical, and coproporphyrin I/III are endogenous compounds. Therefore, we developed validated bioanalytical methods using surrogate matrices.

Methods

In two separate bioanalytical methods, Caffeine and paraxanthine, and coproporphyrin I and III, were extracted from human plasma by ethyl acetate liquid-liquid extraction, and measured by HPLC-MS/MS. 0.9 % bovine serum albumin in 5X phosphate buffered saline was selected as the surrogate matrix.

Data

We confirmed the suitability of the surrogate matrix by assessing precision and accuracy of each analyte spiked into human plasma and quantified against calibration curves prepared in the surrogate matrix. In order to minimize matrix effects, an ethyl acetate extraction was chosen over a more simple protein precipitation method. This cleaner extraction method provided better parallelism between the surrogate matrix and the plasma samples.

The assays were validated with concentration ranges appropriate for expected analyte concentrations based upon historical data from previous clinical trials. The accuracy and precision of each method met FDA guidance. The back-calculated accuracy of the calibrators and QCs was within 15% of the nominal concentration (20% at the LLOQ level), and the %CV was within 15% (20% at the LLOQ level).

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Novel Aspect

Here, we describe the use of surrogate matrices to aid the quantification of important biomarkers of drug-drug interactions using LC-MS/MS.