Abstract

Despite the significant progress in analytical techniques, the development and validation of methods for determining endogenous compounds is still a challenging task due to the natural presence of the analyte in the investigated biological matrix. In contrast to xenobiotics, which do not normally occur in biological material, endogenous substances represent a fundamental problem leading to the impossibility of obtaining an analyte-free sample, the so-called blank, which is used in the development and validation of a new method for the preparation of reference samples. Several generally accepted procedures to solve this problem are described, such as the use of surrogate matrices, analyte-purified matrices, or surrogate analytes; however, the used procedures do not always meet the requirements for developing a reliable analytical method according to the given guidelines and regulations.

This study aimed to propose an alternative approach in the preparation of validation reference samples while preserving the original biological matrix and solving the problem with the natural presence of analyzed compounds in these samples and, subsequently, apply the validated method as part of metabolomic studies focused on the research of selected liver diseases. The presented procedure is based on the standard addition method. However, in contrast to the classic method, the addition is adjusted according to the basal concentration of the monitored substances in the mixed biological matrix, so that a predefined concentration is obtained in the reference samples. In this work, we followed the validation recommendations for bioanalytical methods issued by the European Medicines Agency (EMA). The main advantage and innovation of our validation concept is the implementation of an authentic, unmodified biological matrix and an authentic analytical standard in preparing reference samples. This novel approach was used to validate an LC-MS/MS method designed to quantify 15 bile acids in human plasma. The method was successfully validated with a lower limit of quantification of 5 nM and linearity in the range of 5–2000 nM and used in a metabolomic study on a cohort of 28 pregnant women to detect intrahepatic cholestasis. The research was also focused on bile acids metabolomics in animal models of estrogen-induced cholestasis.