Simple Quantitative Analysis of 40 Total and Fractionated Bile Acids in Serum Using LC-MS/MS for Clinical Research Use

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INTRODUCTION

The bile acids are synthesized in the liver from cholesterol and secreted into the small intestine through the common bile duct as conjugated (with glycine or taurine) and unconjugated forms. They may also be synthesized in the colon from conjugated bile acids. The bile acids act as detergents, emulsifying fats for digestion, and can also serve as precursors for vitamers A and D. The liver secretes into the small intestine 80% of total bile acid pool. In the colon, 90% of bile acids are reabsorbed. In healthy people, a net increase in serum bile acid concentrations is not normally observed. Increased serum bile acid concentrations indicate increased bile acid mass secreted, which can be caused by elevated bile acid synthesis or increased bile acid secretion.

MATERIALS AND METHODS

Standards

The bile acids were purchased from Sigma-Aldrich, USA, and Cerilliant, USA. The standards were used as such, without additional purification. The standards were confirmed by comparison to reference standards. The standards were stored at −20 °C.

EXPERIMENTAL

Method

METHOD

LC/MS Method

The bile acids were separated on a Thermo Scientific Hypersil Gold™ C18 100 x 2.1 mm, 1.8 µm column with a water and acetonitrile/methanol mixture containing 0.1% formic acid as mobile phase A, and 50:50 water and acetonitrile/methanol mixture containing 0.1% formic acid as mobile phase B.

Sample Preparation

Before injection into the LC/MS/MS system, all samples were derivatized with O-anisidine to increase detectability and stability. The derivatization solution was prepared by mixing 100 µL of the derivatization reagent with 400 µL of acetonitrile. A volume of 50 µL of serum was pipetted into a glass vial, 250 µL of acetonitrile was added, and the mixture was vortexed for 1 min. Then, 750 µL of derivatization solution was added, and the mixture was vortexed for 30 s. After vortexing, the sample was left to stand for 1 min, before centrifugation at 15,000 g for 10 min. The supernatant was transferred to a new glass vial.

RESULTS

Table 2 shows the percentage of bile acids and bile acid conjugates detected in serum.

CONCLUSIONS

The bile acids were detected in serum using LC/MS/MS with good sensitivity and specificity. The method was validated for all bile acids and bile acid conjugates. The method was sensitive enough to detect low levels of bile acids and bile acid conjugates in serum.

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REFERENCES


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