Creative Proteomics
SUITE 115, 17 Ramsey Road, Shirley, NY 11967
Tel: 1-631-275-3058 Fax: 1-631-614-7828
www.creative-proteomics.com
info@creative-proteomics.com

Analysis Report

Project Name	Bile acids Quantification Analysis
Sample Description	mouse fecal
Sample Quantity	12
Order Number	CPMS04222402-01
Client	Guillaume Urtecho
Project Date	2024-07
Remark	

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1. Sample Information

12 mouse fecal samples for bile acids quantification analysis in the collected samples;
Sample name: shown in the excel sheet.

2. Sample Preparation and LC-MS Analysis

A mixed solution of 27 targeted bile acids, at 10 μM for each compound, was prepared in a freshly prepared internal standard (IS) solution of 14 deuterium-labeled bile acids. This solution was further diluted step by step to have 10 calibration solutions. Each sample was precisely weighed in a 2-mL homogenization tube. 5 μL of water per mg of raw material was added. The samples were homogenized on a MM 400 mixer mill at 30 Hz for 3 min with the aid of two metal balls. Next, 15 μL of acetonitrile per mg of raw material was added. The samples were homogenized again for 3 min, followed by ultra-sonication in an ice-water bath for 2 min. The samples were centrifuged at 21,000 g for 10 min. 20 μL of the supernatant of each sample was mixed with 180 μL of IS solution. 10 μL aliquots of all the resultant sample solutions and the calibration solutions were injected in turn to run UPLC-MRM/MS. An Agilent 1290 UHPLC system coupled to an Agilent 6495B QQQ mass spectrometer was used. The MS instrument was operated in the multiple-reaction monitoring (MRM) mode and with negative-ion detection. A Waters BEH C_{18} column (2.1*150 mm, 1.7 μm) was used for LC separation and the mobile phase was 0.01% formic acid in water and in acetonitrile for binary-solvent gradient elution.

3. Analytical Results

Linear-regression calibration curves of individual bile acids were constructed with the data acquired from the calibration solutions in appropriate concentration ranges. Concentrations of bile acid detected in the samples were calculated by interpolating the calibration curves of individual bile acids with the analyte-to-internal standard peak area ratios measured from the sample solutions. See attached Excel table for detailed results.