Sample preparation for sulfonate validation measurements

Solvents/Chemicals:

Acetonitrile was purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents for mass spectrometry were of analytical grade purity. Water (resistivity 18.2 MΩcm) was purified using a Milli-Q-System (Millipore, Milford, MA, USA). Formic acid was purchased from Honeywell Fluka (Muskrgon, MI, USA).Cysteic acid and isethionic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). 2,3-dihydroxypropane-1-sulfonate and sulfoquinovose were purchased from MCAT GmbH (Donaueschingen, Germany). Taurine was purchased from Roth GmbH(Karlruhe, Germany). Ti(III) nitrilotriacetate (Sigma-Aldrich, Germany)

Anoxic phosphate-buffered saline (PBS): 8.5 g / l NaCl, 0.3 g / l KH2PO4, Na2HPO4 0.6 g / l, 0.1 g / l Bacteriological Peptone, 1 mg / l Resazurin, 40 mM Sodium DL-lactate , 40 mM Sodium formate pH 7.0, N2/CO2 (80/20, v / v) as gas phase, autoclaved at 121°C for 15 min. all compounds from Fluka ,Muskrgon, MI, USA.

Ti(III) nitrilotriacetate solution: 19.2 g/l nitrilotriacetic acid diluted in anoxic distilled water, pH of 9 adjusted with NaOH,19.2 ml 20% TiCl3 (Acros) pH of 7adjusted with Na2CO3 (80 g/l)all compounds unless specified from Fluka ,Muskrgon, MI, USA.

Work steps for samples for validation measurements

One g of the human fecal sample was homogenized in anoxic phosphate-buffered saline (PBS, pH 7.0, Table 4) by vortexing with glass beads (c. 3 mm; Roth, Germany) to yield a 10% fecal suspension. The fecal suspension was further diluted to 1% in a Hungate tube containing anoxic PBS supplemented with 3.18 mM of sterile filtered Ti(III) nitrilotriacetate (Sigma-Aldrich, Germany) as reductant. Subsequently, fecal slurry aliquotes were centrifuged (14.000 x g, 4°C, 5 min) and the supernatant frozen until further processing. Fecal slurries were s spiked with cysteate, sulfoquinovose (SQ), 2,3-dihydroxypropane-1-sulfonate (DHPS), taurine and isethionate for calibration. fecal supernatants were serially diluted using 50% acetonitrile and 0.1% formic acid in water to obtain final dilution of 1:10,000. 500 µl of each sample were placed in glass vials (Wicom, Heppenheim, Germany) and stored at - 80 °C until LC-MS/MS analysis.