

LC soleus sample preparation protocol

For LC-MS/MS, muscle extracts (50 μ L) were transferred to a glass vial (containing a 250 μ L insert with polymer feet) together with 2.5 ppm (50 μ L) of isotope mixture and evaporated under a stream of nitrogen gas at 37 °C. To derivatize the samples, *N*-butanol:acetyl chloride (200 μ L) was added and the samples were incubated at 50 °C for 1 h. Thereafter the butylated samples were evaporated to dryness under a stream of nitrogen gas at 37 °C. The dried residue was reconstituted in a final volume of water:acetonitrile (50:50) (v/v) containing 0.1% formic acid (25 μ L). Finally, each vial was loaded onto an Agilent© 1200 series auto sampler for LC-MS/MS analysis.