Comparative Analysis of Metabolite Extraction Protocols in Nile Tilapia Plasma

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Environmental metabolomics explore how organisms react to different environmental stimuli. In ecotoxicology, changes in the metabolic profiles of aquatic species could predict how pollutants affect higher levels of biological organization, aiding in evaluating ecosystem health and identifying new threats. For this, preparing and extracting metabolites from chosen biological matrices is vital in metabolomics studies. Given their diverse chemical properties — such as polarity, solubility, and volatility — specific extraction methods are needed to capture a broad spectrum of compounds. Effective extraction methods enhance the detection of metabolites present in low concentrations, crucial for understanding biological processes or identifying biomarkers. This study aimed to compare two extraction protocols for metabolites from Nile tilapia (*Oreochromis niloticus*) plasma, a key fish species in Brazil. In protocol I (P1), 200 µL of plasma added to 1 mL of chilled methanol and 10 µL of internal standard (sulfonated methionine) were vortexed for 5 minutes. They were then centrifuged at 13,000 G at 4 ºC for 15 minutes, and a 1mL aliquot of supernatant was separated. In protocol II (P2), 200 µL of plasma added to 600 µL of a chilled methanol:water mixture (3:1) and 10 µL of internal standard were vortexed for 30 seconds, and then an additional 450 µL of chilled chloroform was added. This mixture was left in an ice-cooled ultrasonic bath for 10 minutes. They were then centrifuged at 10,000 rpm at 4 ºC for 10 minutes. Finally, aliquots of 700 µL of the hydrophilic phase and 400 µL of the hydrophobic phase were separated into different microtubes. All samples were dried under nitrogen and reconstituted in 400 µL of methanol, then analyzed using high-performance liquid chromatography and high-resolution QTOF mass spectrometry on a C18 column. Samples were divided into six groups according to their origin and the protocol used, and a 75% frequency filter was Applied. This revealed: 32 metabolites unique to P1, 24 to P2, and 34 common to both. P1 detected various aromatic compounds, amino acids, and acid derivatives. P2 found vitamin derivatives, complex lipids, glycolipids, and rarer compounds. Shared metabolites included fatty acids, amino acids, and glycolipid compounds, vital for biological functions like membrane structure and metabolic processes. Based on the polarity of the compounds, both protocols allowed the detection of both polar (LogP < 3) and nonpolar (LogP > 3) compounds. A slightly higher number of polar compounds was observed in P1. However, the same number of nonpolar compounds, in the same range (LogP > 3 to 17), was detected in both. The protocols differed in chemical diversity and compound focus. The choice between one or the other depends on the objective of the study. Protocol 1 allows the detection of a wider range of compounds, especially polar compounds. Meanwhile, some lipids were only detected in P2.

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