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BOOK OF ABSTRACTS

OPTIMIZATION OF SAMPLE PREPARATION FOR QUANTIFICATION OF β-ALANINE AND CARNOSINE IN MUSCLE TISSUE BY LC-MS/MS

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β-Alanine and carnosine are bioactive compounds involved in muscle physiology, commonly studied in exercise biochemistry and metabolic research. However, variability in reported outcomes suggests that supplementation may not be effective for all individuals, justifying the need for studies that identify determinants of a positive response. The quantification of these substances in biological samples is fundamental step for the evaluation of the response of each individual. Reliable quantification of these analytes in muscle tissue requires sensitive and selective methods such as liquid chromatography coupled–tandem mass spectrometry (LC-MS/MS). However, sample preparation remains a critical step that can significantly impact analytical performance, especially in complex biological matrices like muscle. This study aimed to optimize a sample preparation protocol to quantify β-alanine and carnosine in muscle by LC-MS/MS. Different sample preparation variables were evaluated, including extraction solvents (acetonitrile, hydrochloric acid and perchloric acid), temperature control, and tissue-homogenization strategy (with or without liquid-nitrogen freezing). Optimization was guided by peak shape, analyte peak area, and signal-to-noise ratio. Chromatographic separation was performed using an Acquity UPLC BEH HILIC (100 × 2.1 mm, 1.7 µm) column, maintained at 30 °C under gradient elution. Detection was carried out by ESI positive and in multiple reaction monitoring (MRM) mode. Acidic extraction under temperature control, combined with liquid-nitrogen freezing, yielded higher peak areas. Moreover, the use of $HClO_4$ for extraction allowed the detection of β-alanine also from bovine samples. Freezing with liquid nitrogen proved to be a critical step during the experiments. The optimized sample preparation protocol enables the detection of β-alanine and carnosine in bovine and poultry muscle by LC-MS/MS. Next steps include validating the protocol in human muscle samples.

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