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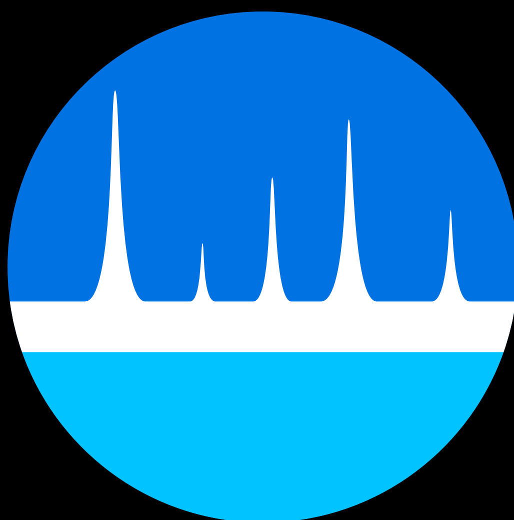

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LIVRO DE RESUMOS

BOOK OF ABSTRACTS

Development and Optimization of a LC-MS/MS Method for Determining Xanthines in Plasma Samples

Maria Eduarda Diniz*[1], Taís Betoni Rodrigues[1], Álvaro José Santos-Neto[1], Bryan Saunders[2]

[1]Laboratory of Chromatography (CROMA), São Carlos Institute of Chemistry, University of São Paulo, São Carlos, São Paulo, Brazil
[2]Faculty of Medical Sciences, University of São Paulo, São Carlos, São Paulo, Brazil

* Corresponding author: mariaeduaradadiniz2@usp.br

Regular physical activity is often associated with nutritional supplements aimed at improving performance, recovery, or muscle gain. Adequate supplementation can enhance athletic performance by optimizing muscle function and exercise tolerance. Among the substances studied, caffeine stands out as one of the most widely used in sports. However, the variability of results suggests that supplementation is not equally effective for all individuals, reinforcing the need for research to identify the determining factors for positive effects. In this context, the quantification of caffeine and its metabolites in biological matrices is essential for assessing individual responses. Therefore, this study aimed to develop an LC-MS/MS method for their determination in plasma samples. Plasma samples (100 µL) were subjected to protein precipitation with cold acetonitrile (400 µL) in the presence of an internal standard (caffeine-d9, 500 ng mL^{-1}). After vortexing and centrifugation (14,000 rpm, 10 min), the supernatant was dried in a SpeedVac (60 °C, 1h20). The residue was resuspended in H_2O:MeOH (90:10, v/v), centrifuged again, and the supernatant transferred to a vial with an insert. Then, 3 µL was injected into the LC-MS/MS. Analyses were performed on an ACQUITY UPLC system coupled to a Xevo TQ-MS triple quadrupole mass spectrometer (Waters, USA), equipped with an Acquity UPLC BEH C18 column (100 × 2.1 mm, 1.7 µm) and Vanguard pre-column (1.7 µm), maintained at 40 °C. The mobile phases were: (A) water + ammonium formate (5 mmol L^{-1}) and (B) methanol, with a flow rate of 0.3 mL min^{-1}, under a 15–95% B gradient, totaling an 8-min run. Detection was performed by ESI(+) in MRM mode. Data control, acquisition, and processing were performed using MassLynx software (Waters, USA). As a result, a concise and robust method for the analysis of xanthines was developed. However, column clogging was recurrent during method development, even with the use of a Vanguard pre-column, suggesting that the issue is related to sample preparation. Since the current protocol does not include lipid and phospholipid removal, an Ostro 96-well plate was acquired to assess whether the accumulation of these components is affecting chromatographic analysis of xanthines.

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