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Development of a methodology using GC-FID for the quantitative analysis of fatty acids from red blood cells

Helena Costa[1], **Raquel Rodrigues**[1,2], **Rui Lima**[1,3,4], **Joana S. Amaral** [1,5*]
[1] ESTiG - Instituto Politécnico de Bragança
[2] LCM - Laboratório associado LSRE/LCM, Faculdade de Engenharia da Universidade do Porto
[3] Universidade do Minho, Departamento de Engenharia Mecânica, Guimarães
[4] CEFT/FEUP, Universidade do Porto, Porto
[5] REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia da Universidade do Porto
*jamaral@ipb.pt

In the last years, there has been an increasing interest in evaluating possible relations between fatty acid patterns and the risk for chronic diseases. Currently, it is generally accepted that higher intakes of saturated and trans fatty acids are related with dyslipidemia and increased risk for cardiovascular diseases, while the consumption of polyunsaturated fatty acids, especially omega-3, has been positively associated with several health benefits. So far, most studies concerning the analysis of blood fatty acids (FA) composition have been performed using plasma or serum, with red blood cells (RBCs) usually being discarded [1]. However, because of the long half-life (120 days) of these cells, the FA profile of RBCs membrane may reflect longer-term markers of nutritional intake compared with plasma or urine [1]. Furthermore, it has also been suggested its use as an appropriate biomarker to investigate patterns and possible correlations between FA metabolism and diseases [2, 3]. The aim of the present work was to develop a simple and fast method for the identification and quantification of FA present in RBCs membranes using gas chromatography with flame ionization detector (GC-FID). For this purpose, RBCs were isolated from venous blood samples and added with BHT as antioxidant. Then, different sample preparation protocols were tested including a classic two-step method (Folch method) with modifications and different one-step methods for which lipid extraction and derivatization where performed in a single step. For the one-step methods, different parameters, such as methylation period and the inclusion of saponification reaction, were evaluated. Fatty acid methyl esters (FAMEs) analysis was performed on a Bruker® SCION 436-GC with a FID and a CP-Sil 88 column (50m x 0.25mm i.d, 0.20μm, Agilent J&W). Fatty acids were identified using a mixture of 37 FAMEs (Supelco) and C19:0 was used as internal standard. Based on the obtained results, the one-step method with saponification and 60 min methylation was selected as being the most suitable for the intended purposes, allowing the identification of a higher number of FAMEs.

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