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A REVERSED-FLOW MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY METHOD USING CENTRAL COMPOSITE DESIGN FOR THE FINGERPRINT ANALYSIS OF Chrysobalanus icaco

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Chrysobalanus icaco is a medicinal plant used in Brazil for controlling blood glucose levels in diabetic patients. There are promising results regarding the pharmacological effect observed when testing extracts of the plant leaves [1]. However, the content of the secondary metabolites found in this medicinal plant are susceptible to environmental factors [2]. Thus, the fingerprint analysis is an efficient alternative for the authentication and quality control of this medicinal plant [3]. Such metabolites are obtained from the plant by an optimized procedure yielding a consistent separation when analyzed by a reversed-flow micellar electrokinetic capillary chromatography (RF-MEKC) method. To obtain a more reliable fingerprint of the metabolites, it is important to maximize the number of peaks observed. In this context, this work aims to develop a capillary electrophoresis fingerprint method for the extract of C. icaco using a 70° GL (Gay-Lussac degrees) experimental protocol. A central composite design (2^2) has been carried out to study the effect of surfactant and background electrolyte concentrations on the number of peaks detected. The RF-MEKC separation conditions were developed using a phosphate buffer as running buffer containing sodium dodecylsulfate (SDS) at pH 2.5. Samples were introduced into the capillary at 25 mbar for 6 s and separated using a separation voltage of -20 kV and a temperature of 20 °C. The experiments were performed employing an Agilent 3D capillary electrophoresis system equipped with a diode-array detector, using a wavelength of 254 nm for monitoring the separated metabolites. The dimensions of the capillary were 58.5 cm in total length, 50 cm to the detector, and 50 µm i.d. The results indicated that the number of peaks was higher when using 50 mmol/L phosphate buffer containing 16 mmol/L SDS. Even though the analysis of variance (ANOVA) showed good results, they cannot be considered satisfactory for the purpose of generating a predictive model. The contour plot indicated that the trend of the optimum condition of separation was obtained when using a phosphate buffer in the region below the concentrations of 80 mmol/L phosphate and 20 mmol/L SDS. In this way, a new experimental design must be performed to assess the interval between 20 mmol/L SDS and its critical micelle concentration (8.2 mmol/L). We concluded that the method described in this study was useful to establish the best parameters for the fingerprint of the metabolites of the medicinal plant C. icaco.

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