

**OPTIMIZATION OF AN ECOLOGICAL ANALYTICAL PROCEDURE FOR  
METABOLIC PROFILING *Theobroma grandiflorum* AND *Theobroma cacao***

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The *Theobroma* spp. is composed of a group of fruit trees native to the Amazon with great economic and cultural relevance. *T. cacao* L. (cocoa) stands out as it is widely cultivated in Brazil and used in the food, cosmetic, and pharmaceutical industries, followed by *T. grandiflorum* (cupuaçu)<sup>[1]</sup>. This work aimed to develop a highly efficient and green analytical procedure for analyzing parts of these species from a multivariate approach. The separation by UHPLC-DAD/UV-MS was optimized from a four-factor Doehlert design, while the extraction was from a two-factor central rotational compound design from a mixture of cupuaçu parts (leaf, husk, seed, and pulp). An optimal separation ( $R^2 = 0.85$ ) was achieved with 5% of 50% MeOH in 20 min, using 0.2% HCOOH in the mobile phase, which resulted in  $80.5 \pm 2.4$  peaks at  $\lambda = 280$  nm. Then, an optimal extraction ( $R^2 = 0.80$ ) was achieved using dynamic maceration with 30% ethanol for 60 min, resulting in  $88.8 \pm 2.8$  peaks at  $\lambda = 280$  nm. The full optimized condition, from sample preparation to separation, was applied to each part of the cupuaçu separately. Leaves had the highest number of peaks ( $85.0 \pm 0.0$ ), followed by husks ( $80.5 \pm 0.7$ ), seeds ( $65.5 \pm 2.1$ ) and pulp ( $42.0 \pm 2.8$ ). For comparison, the developed method was also satisfactorily applied to different parts of cocoa, resulting in  $83.0 \pm 2.8$ ,  $59.5 \pm 2.1$  and  $70.0 \pm 1.4$  in leaves, husks and seeds, respectively. The adopted multivariate approach led to a high level of chemical information for individual parts of both cupuaçu and cocoa, while employing only recommended solvents<sup>[2]</sup>. This globally optimized analytical procedure will be applied for compound annotation using UHPLC-QToF-MS/MS. The authors would like to thank CNPq (131186/2024-8 and 03675/2021-7) and CAPES (88887.172509/2025-00).

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