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Extraction optimization, development and validation of a new DAD-UHPLC quantitative method for 20-hydroxyecdysone in *Sida tuberculata* leaves

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Sida tuberculata species, Malvaceae, popularly known as “guanxuma”, is widely distributed in southern Brazil. The genus is used in folk medicine due to their biological properties as hypoglycemic, anti-cholesterol and antimicrobial. The plant is chemically characterized mainly by flavonoids, alkaloids and ecdysteroids.^{1,2} The present work aimed to optimize the extractive method and to validate a new DAD-UHPLC method for the quantification of 20-hydroxyecdysone (20-HE) in leaves of the species. *S. tuberculata* leaves, were collected in Uruguai, RS, and the optimization of extractive method was performed in two steps (extractive method selection and optimization by Box-Behnken), through the statistical software MiniTab 17®. The extractive methods tested were static and dynamic maceration, ultrasound, ultra turrax and reflux. In the Box-Behnken (DBB) model the parameters were evaluated in three levels (-1, 0, +1): granulometry, time and drug:solvent ratio.³ In the validation method, the parameters selectivity, specificity, linearity, limits of detection and quantification (LOD, LOQ), precision, accuracy and robustness were evaluated.⁴ The results pointed out for static maceration as the better extractive method, while the optimal extraction from DBB was obtained with these parameters: granulometry of 710 µm, 9 days of maceration and drug:solvent ratio of 1:54 (w/v). Selectivity was proved by the addition of a reference substance in the sample solution, which produced an increase only in the peak area correspondent to the compound. Specificity was verified by comparing UV profiles of reference and sample solutions. Linearity was determined with reference solutions in concentrations of 10.42-93.74 mg/ml, with $r^2 = 0.9995$. LOD and LOQ were 2.63 and 7.97 µg/ml, respectively. Repeatability and reproducibility presented adequate results, with RSD of 4.29% and 2.93%, respectively. The mean recovery level for accuracy was 95.40%. Robustness was evidenced by changes in flow, analytical column and wavelength; all results showed RSD of less than 5%. The average content of 20-hydroxyecdysone was 0.56%. Thus, the optimization of extractive method in *S. tuberculata* leaves increased the concentration of 20-hydroxyecdysone in crude extract and the performed method is in accordance to the validation requirements of the current legislation.

Keywords: *Sida tuberculata*, 20- hydroxyecdysone, method validation.

References

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