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## **Optimization of the extraction of rosmarinic acid from *Blechnum brasiliense* leaves, development and validation of analytical method for the quantification**

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*Blechnum brasiliense* Desv., Blechnaceae, is a fern characterized by the major presence of phenolic compounds, especially rosmarinic acid (RA), and present several biological activities, such as neuroprotective, antioxidant, anti-inflammatory, antimicrobial and antitumor.<sup>1,3,5</sup> In this work, we performed the optimization of a extraction method, as well as the development and validation of analytical method for quantification of RA, by Ultra Performance Liquid Chromatography (UPLC), in the *B. brasiliense* leaves. Plant material was collected in the southern region of Brazil and the extraction optimization was performed in three stages: extractive method selection; screening using Fractional Design Factor (FFD) and Box-Behnken Design (BBD) optimization, using MiniTab 17<sup>®</sup> software for statistical data processing.<sup>2,4</sup> Four extractive methods were tested: ultraturrax, ultrasound bath, static maceration and reflux bath. FFD evaluated four factors at two levels: hydroethanol solution, drug:solvent ratio, particle size and extraction time. For BBD analysis, the time was fixed and the other parameters were reassessed in three levels. The obtained extracts were analyzed by UPLC and the area of the AR peak in chromatograms was evaluated. The method was validated by checking the specificity / selectivity steps, linearity, detection and quantification limits (LOD and LOQ), precision, accuracy and robustness. The extraction method selected was reflux (15 min/90 °C). Results of FFD showed influence of all the parameters, being the best answers obtained using shortest time and proportion of hydroethanolic solution and greater particle size and drug:solvent ratio. The BBD results showed as excellent extraction parameters: particle size of 1400 µm, hydroethanol solution at 28% (v/v) and drug:solvent ratio 1:64 (m:v), for the predicted area of the RA peak equal to 6238574. The method was selective / specific, linear in a range of RA concentration from 10 to 80 µg/ml, LOD and LOQ equal to 3.044 and 9.225 µg/ml, respectively. The precision and accuracy steps were verified, with mean RA content of 0.253% and a recovered average RA content of 100.7% respectively, both with relative standard deviation (RSD) of less than 5%. The method showed robustness, since the parameters tested did not demonstrate RSD higher than 5% in relation to the normal results of analysis. The extractive methods and parameters evaluated were able to increase the amount of RA obtained in the crude extract of *B. brasiliense* and, according with the statistical analyzes, it was possible to select the optimal conditions for extract this compound, obtaining a rosmarinic acid enriched extract.

**Key words:** *Blechnum brasiliense*; Rosmarinic acid; UPLC method validation.

### Referências

- <sup>1</sup>Andrade, J.M.M., Passos, C.S., Kieling, R.M.A., Mendonça, J.N., Lopes, N.P., Henriques, A.T., 2016. Combining *in vitro* and *in silico* approaches to evaluate the multifunctional profile of rosmarinic acid from *Blechnum brasiliense* on targets related to neurodegeneration. Chem-Biol. Interact. 254, 135-145.



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- <sup>2</sup>Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escalera, L.A., 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta* 76, 965-977.
- <sup>3</sup>Franz, I., Schmitt, E.J.L., 2005. *Blechnum brasiliense* Desv. (Pteridophyta, Blechnaceae): estrutura populacional e desenvolvimento da fase esporofítica. *Pesq. Bot.* 56, 173-184.
- <sup>4</sup>Kaiser, S., Verza, S.G., Moraes, R.C., Pittol, V., Peñaloza, E.M.C., Pavei, C., Ortega, G.G., 2013. Extraction optimization of polyphenols, oxindole alkaloids and quinovic acid glycosides from cat's claw bark by Box-Behnken design. *Ind. Crop. Prod.* 48, 153-161.
- <sup>5</sup>Petersen, M., Simmonds, M.S.J., 2003. Molecules of interest - rosmarinic acid. *Phytochemistry* 62, 121-125.