5th BCNP Brazilian Conference on Natural Products
XXXI RESEM
Atibaia – SP – Brazil
ISBN: 978-85-66836-10-3
Oct. 26–29th 2015

DEVELOPMENT AND VALIDATION OF A HPLC-DAD ANALYTICAL METHOD FOR *Copaifera* OLEORESINS DITERPENES

Luiza J. Carneiro[1], Jonas Mangabeira[2], Rodrigo C. S. Veneziani[1], Jairo K. Bastos[2], Sérgio R. Ambrósio[1]

[1]Núcleo de Pesquisas em Ciências Exatas e Tecnológicas, Universidade de Franca, Franca, Brazil; [2]Departamento de Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto – USP, Ribeirão Preto, Brazil; junqueira_ljc@hotmail.com

The oleoresin obtained from *Copaifera* species has been largely used in folk medicine and several studies have demonstrated its pharmacological potential, specially as anti-inflammatory, wound healing and anticancer [1,2]. In Brazil, these oils are extensively commercialized and exported for use in pharmaceutical and cosmetic preparations [1]. To ensure the authenticity of plant material and the quality of herbal medicine, the presence and proportion of its chemical markers should be analyzed [3]. Analytical methodologies using gas chromatography for the characterization and quantification of *Copaifera* oleoresins have already been described; however, these methods are more adequate to analyze volatile compounds [1,4]. Considering this, a HPLC-DAD method based on the main diterpenes of these oleoresins was developed and validated for three *Copaifera* species: *C. multijuga*, *C. duckei* and *C. reticulata*. Initially, the volatile portions of the oleoresins were separated by hydrodistillation from the fixed portions, which were used for the method development. The developed method established the use of an isocratic elution system of 80% acetonitrile in water with 1% acetic acid at a flow rate of 1.0 mL/min. The temperature of the column was 40ºC and the detection was carried out at 201 nm. Manool was used as internal standard. The developed method was validated by analyzing the selectivity, linearity, limits of detection (LOD) and quantification (LOQ), precision, accuracy and robustness, according to Moreira, et al. (2013). The UV spectral purity of each diterpene peak confirmed the selectivity of the method. Linearity analysis showed correlation coefficients (r^2) above 0,99, and low LOD and LOQ values. When evaluated the precision and accuracy of the method, the relative standard deviation (RSD) values for diterpenes concentrations were less than 5%, and the means obtained on different days did not differ statistically ($p < 0.05$). Robustness analysis showed RSD values below 20%. In conclusion, these results evidence that the RP-HPLC method developed is suitable for use in different labs.

[1] Souza, A.B., Moreira, M.R., Borges, C.H.G., Simão, M.R., Bastos, J.K., De Sousa, J.P.B., Ambrósio, S.R. and Veneziani, R.C.S. 2013. Development and validation of a rapid RP-HPLC method for analysis of (-)-copalic acid in copaiba oleoresin. Biomedical Chromatography. 27: 280-283.

[2] Gomes, N.M., Rezende, C.M., Fontes, S.P., Hovell, A.M., Landgraf, R.G., Matheus, M.E., Pinto, A.C. and Fernandes, P.D. 2008. Antineoplasic activity of *Copaifera multijuga* oil and fractions against ascitic and solid Erlich tumor. Journal of Ethnopharmacology. 119: 179-184.

[3] Schaneberg, B.T., Crockett, S., Bedir, E. and Khan, I.A. 2003. The role of chemical fingerprinting: application to *Ephedra*. Phytochemistry. 62: 911-918.

[4] Moreira, M.R., Souza, Ariana B., Moreira, Maísa A., Bianchi, T. C., Carneiro, L. J., Estrela, F. T., Dos Santos, R. A., Januário, A. H., Martins, C. H. G., Ambrosio, S. R. and Veneziani, R. C. S. 2013. RP-HPLC analysis of manool-rich Salvia officinalis extract and its antimicrobial activity against bacteria associated with dental caries. Revista Brasileira de Farmacognosia. 23: 870-876.