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

RESPONSE SURFACE METHODOLOGY (RSM) APPLIED TO EXTRACTION OPTIMIZATION TOWARDS PLANT METABOLOMICS

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Extraction is an underlying step in analytical approaches for plant metabolomic studies and natural products libraries generation. In order to obtain a representative sample is necessary to perform the extraction in fully optimized conditions [1,2]. In this context, we applied chemometric tools to evaluate the effects of extraction parameters and establish the optimal condition. A multivariate regression method named Central Composite Design (CCD) was proposed to fit a model to explain important plant extraction processes parameters. The goal was to achieve the maximization of the following responses: number of peaks and total area of peaks in LC-MS analysis [3,4]. The experimental part used a three-factor and rotatable CCD consisting of five levels and nineteen experiments with five replicates at the central point. The following variables were selected based on evidences from previous experimental screenings and literature: solid-to-liquid ratio (5:1-20:1, m:v; X_1); ethanol proportion (40-90%, EtOH:H_2O, X_2) and time of ultrasound-assisted maceration (10-30 min; X_3). Variable coding and modelling were performed in *Excel* and *R* softwares. Assessment of significance to each variable and prediction capability of the proposed models were performed using ANOVA, *F*-tests and *t*-tests. Leaves from 45 species from different genera belonging to Asteraceae family were dried, powdered using liquid nitrogen, mixed and extracted by ultrasound-assisted maceration (40 MHz). Experiments were randomly prepared (three replicates) and analyzed by UHPLC-HRFTMS (Thermo Scientific Exactive Plus™, equipped with Orbitrap™ technology from Thermo Scientific©). A first-order polynomial model without lack of fit (Fcalc < Ftab) was obtained for the response "number of peaks": $Y = 919.8 + 117.5X_1$ (R = 0.66). For the second response studied "total peak area" a second-order polynomial model was obtained: $Y = 0.544 + 0.244X_1 + 0.187X_{12}$ (no lack of fit, Fcalc < Ftab, R = 0.78). For both responses the important design variables must be fixed at high levels and the optimum points obtained after observing the surface obtained (Response Surface Methodology, RSM) confirmed that. The optimal extraction condition determined by overlapping of both surfaces were: solid-to-liquid ratio of 20:1 (m:v); 80% of ethanol and 20 min of ultrasound-assisted maceration. The obtained results highlights that, by itself, the only parameter that effectively influenced the maximization of both desired responses was solid-to-liquid ratio, fact that made unnecessary a desirability approach to identify a suitable commitment condition. However, to the "total peak area" response the interaction of solid-to-liquid ratio and ethanol proportion was also significantly important.

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