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

MULTIVARIATE CHOMATORAPHIC PARAMETERS SELECTION FOR THE ETHANOLIC EXTRACT OF BAUHINIA *SPP.*

Aquino A. J.*, Pereira-Filho E. R, Cass Q. B.

*Chemistry Department, Federal University of São Carlos, Brazil. (*jord_amanda@yahoo.com.br)*

In search for bioactive compounds, crude plant extracts is a most productive source of molecules that can furnish lead compounds for drug discovery. To meet this end, liquid chromatography (LC) hyphenated to MS/MS or to NMR has been efficiently used as a mean of obtaining direct structural information. The selection of the chromatographic parameters by univariate approach is, however, the main drawback in the development of these analytical procedures. This study reports the selection of chromatographic parameters by multivariate approach for the analyses of ethanolic extracts of Bauhinia *ssp* (*forficata*, *variegata* and *longifolia*).

The chromatographic conditions were all evaluated in reverse-phase mode at 40° C with a flow rate of 0.3 for the fused-core columns and of 1 mL/min for the porous particle column. To improve ionization, 100 μM of formic acid were used. The analyses were carried out by LC-MS/MS. The examined variables were: gradient time (40 for core-shell or 60 min for Restek, level -1 and 60 for core-shell or 90 min for Restek, level 1) and solvent used: methanol (level -1) or acetonitrile (level 1) as organic modifier (2^2 = 4 experiments). Each set of experiments (4) were carried out in four different orthogonal stationary phases: Biphenyl – Kinetex, Biphenyl – Restek, Ascentis Express F5 and Ascentis Express C18. A total of 24 experiments were performed (some of the in authentic replicates) and for each stationary phases a linear model was calculated using Excel and Matlab software at α confidence level ≤ 0.05 [1]. The observed response (y) was the number of chromatographic bands with signal to noise ratio (S/N) higher than 10.

For all species analyzed a model was obtained and evaluated with ANOVA (Analysis of Variance). Table 1 shows the significant coefficients. In general the Restek column presented the best results (high number of separate peaks). Thus, as the objective is dereplication, i.e. find different compounds in the species them with different retention times, were chosen the same and more restrictive condition in which all linear variables are in the level -1.

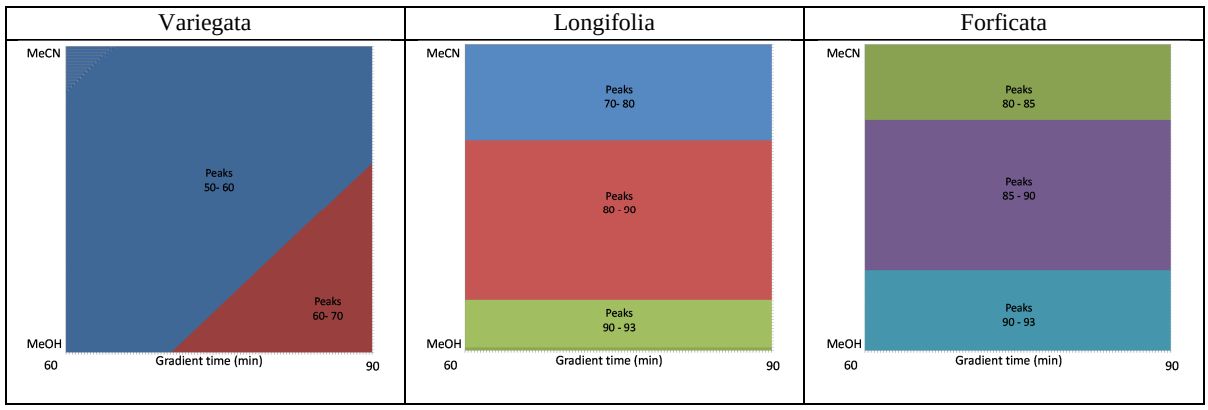
Table1: Linear models for the species analyzed

Columns	***forficata***	***variegata***	***longifolia***
Kinetex	y = 58 + 7S	y = 32 + 5G + 5S + 9GS	y = 55 + 7G
Restek	y = 88 - 5S	y = 57+4G-4S	y = 83
AEF5	y = 47 + 4G - 7,5S	y = 36 + 4G - 3GS	y = 51 + 7G - 8S - 3GS
AEC18	y = 53 + 6G	y = 31+4G	y = 51 + 7G

***S = organic modifier; G = gradient time; GS = gradient time x organic modifier

Figure 1: Comparison between gradient time and organic modifier for column Biphenyl – Restek ®

ISBN: 978-85-66836-10-3



Although columns Kinetex and Restek biphenyls are chemically similar, that showed very different results. Probably was due to the extra-column volume, because superficially porous columns have maximum efficiency UHPLC equipment than LC. [5]

Moreover, bauhinia *ssp* are known to have a variety of phenolic secondary metabolites and thus justifies the selection of a biphenyl stationary phase as the one that furnished the highest number of peak. This can be explained through the π- π interactions. The use of acetonitrile impairs these interactions worsening the separation.

References

[1] PEREIRA FILHO, E. R. **Planejamento fatorial em química: maximizando a obtenção de resultados**. EDUFSCar. Série apontamentos, p-88, 2015.

[2] SNYDER, L. R. A New Look at the Selectivity of RPC Columns. **Analytical Chemistry,** v.79. n. 9, p. 3254-3262, 2007.

[3] TAYLOR, T. HPLC Column Transfer from Fully Porous to Core–Shell Particles in Three Simple Equations. **LCGC - solutions for separation scientists,** v. 11, n. 9, p. 2-4, 2015

[4] FERRERES, F. et al. Bauhinia forficata Link authenticity using flavonoids profile: relation with their biological properties. **Food Chem,** v. 134, n. 2, p. 894-904, 2012.

[5] GUILLARME D., F. S. Fast and efficient HPLC separations with superficially porous particles. **Perkin Elmer**, p. 9, 2013.