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

Identification of Natural Products in complex mixtures: a deconvolution and clustering strategy based on RANSY, DOSY and 1D ^{1}H-TOCSY

Alan Cesar Pilon[1], Fausto Carnevale Neto[2], Rafael Teixeira Freire[1], Patrícia Cardoso[1], Ian Castro-Gamboa[1] and Vanderlan da Silva Bolzani[1]

[1]Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais, NuBBE, Instituto de Química, Universidade Estadual Paulista (UNESP), Araraquara, SP, Brazil; [2]Núcleo de Pesquisa em Produtos Naturais e Sintéticos (NPPNS), Faculdade de Ciências Farmacêuticas de Ribeirão Preto da Universidade de São Paulo (FCFRP-USP), Ribeirão Preto, SP, Brazil;

pilonac@gmail.com

Due to the chemical complexity of natural products, characterization of bioactive compounds in extracts usually requires time-consuming multistep purification procedures to achieve structural elucidation of individual metabolites. In this work we developed a dereplication strategy for in situ identification of compounds in complex mixtures. The strategy is based on variation of metabolites concentrations by HPLC-DAD-SPE-NMR system. Once the extract is fractionated and analyzed by NMR, the spectra of each pure metabolite is recover based on RANSY [1] and STOCSY [2] statistical methods by means of ^{1}H signal intensity variation. To illustrate application of this strategy, methanol extract from leaves of *Solanum paniculatum* (Solanaceae) was analyzed by HPLC-DAD-SPE [3]. Fractions were collected in (GP) cartridges after each 80 seconds, dried and transferred to 3mm NMR tube. RANSY and STOCSY tools were used to deconvolute substances present in three subsequent fractions. DOSY, 1D ^{1}H-TOCSY, HSQC, HMBC and H-H COSY experiments were performed in order to validate the deconvoluted NMR signals. A list of chemical shifts was assigned to each substance and 1D ^{1}H-TOCSY experiments were performed revealing all protons associated to each spin system. Additionally, HSQC and HMBC were performed in order to concatenate H-C bonds as well as determine bonding points among different spin system. The ^{1}H NMR spectrum generated by RANSY and STOCSY (spin-correlated system) combined with DOSY experiments and 1D ^{1}H-TOCSY allowed to determine six spin systems associated to triterpene within amatrix containing six compounds. Integrating 1D 1H-TOCSY, HSQC and HMBC data led to selective dereplication of a glycoside steroidal triterpene, paniculin A, already reported from *Solanaceae paniculatum*.

[1] WEI, S.; ZHANG, J.; LIU, L.; YE, T.; NAGANA GOWDA, G.A.; TAYYARI, F.; RAFTERY, D. Ratio Analysis NMR Spectroscopy (RANSY) for Selective Metabolite Identification in Complex Samples. Anal Chem., v. 83, n. 20, p. 7616–7623, 2011.

[2] CLOAREC, O.; DUMAS, M.E.; CRAIG, A.; BARTON, R.H.; TRYGG, J.; HUDSON, J.; BLANCHER, C.; GAUGUIER, D.; LINDON, J.C.; HOLMES, E.; NICHOLSON, J. Statistical total correlation spectroscopy: an exploratory approach for latent biomarker identification from metabolic 1H NMR data sets. Anal Chem., v. 77, n. , p. 1282-1289, 2005.

[3] GÓMEZ-ROMERO, M.; SEGURA-CARRETERO, A.; FERNÁNDEZ-GUTIÉRREZ, A. Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. Phytochemistry, v. 71, n. 16, p. 1848-1864, 2010.

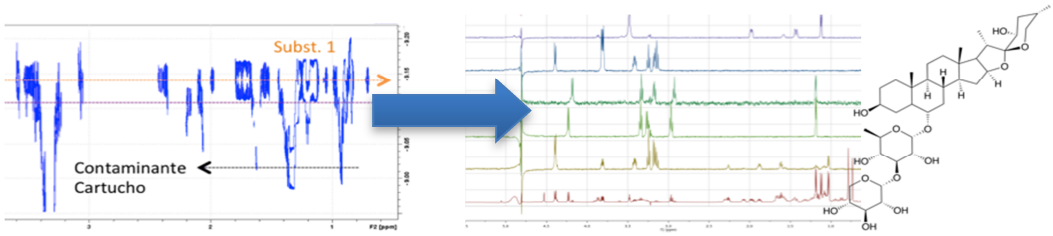

Figure 1. Dereplication of steroidal triterpene based on a deconvolution and clustering strategy based in RANSY, STOCSY, DOSY and 1D ^{1}H-TOCSY.