ANALYSIS OF ORGANOPHOSPHATE RESIDUES IN PROPOLIS EXTRACTS BY AN MSPD APPROACH WITH GC-FPD DETERMINATION

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Propolis extracts for pharmaceutical and food use are a dense mass composed by polyphenols and resins obtained after removing waxes or insoluble material with ethanol from the raw material that was scrapped from the hive [1]. Although an insect-derived-product, pesticides residues in propolis are common and arise from two main sources, either from contamination due to agricultural practices or to pesticide application in the hive, to prevent parasitic acaroids like *Varroa destructor* or ants. Acaricides like coumaphos are widely employed in apiculture and their residues are found in Propolis. They must be currently controlled, in order to establish rules in propolis production management and to determine the quality of extracts, although neither official method, nor regulation is given for pesticide residues in propolis, due perhaps, to matrix complexity. To achieve adequate extraction and clean-up steps during sample preparation are key issues for propolis pesticide residue analysis.

In the present communication, we report the analytical features of a novel method for organophosphate pesticides (coumaphos, chlorpyriphos and ethion) residue analysis based on MSPD followed by SiO_2 column chromatography purification applied to ethanolic propolis extracts.

Direct dispersion of acetonic solutions of 8% (w/v) onto anhydrous $Al_2(SO_4)_3$. is subjected to an MSPD approach to remove most phenolic compounds and water from extracts. The solid mixture is then conditioned in a cartridge using Florisil as co-column material. A first raw extract is obtained after elution with 30 mL of AcOEt:CH_2Cl_2 (1:9) mixture.

The solvent is concentrated under reduced pressure and to dryness with mild N_2 stream. Subsequent clean-up is performed by normal phase column chromatography using CH_2Cl_2. After solvent evaporation, pesticide determination is achieved with a GC-FPD analytical system using bromophos methyl as internal standard.

Recovery study at three levels (from 0.2 to 2.0 mg/Kg) shown percentages for the selected pesticides ranging from 74.1% to 103.9% with RSD<16.2. Matrix effect was evaluated and matrix match calibration was chosen for quantitation purposes as a strong matrix effect for chlorpyriphos and ethion was obtained in all the dynamic range (0.05 to 4.0 mg/Kg).

LODs and LOQs ranged from 0.015 and 0.048 mg/Kg for coumaphos, 0.017 and 0.050 mg/Kg for chlorpyriphos and 0.021 and 0.62 mg/Kg for ethion respectively.

Reproducibility and performance with respect to precision was evaluated with HorRat index [2] resulting in excellent to satisfactory values of consistence from 0.10 to 0.78.

Over 900 real samples from Uruguay were processed to date with the developed method. Coumaphos residues were identified in most samples, ranging from unnoticeable to 45.5mg/Kg although small amounts of chlorpyriphos, and traces for ethion residues were founded in some samples and were associated to environmental contamination.

This method provides a suitable methodology for routine analysis of *Varroa sp.* controllers commonly used in Uruguay and others organophosphate residues from contamination sources without interferences.

[1] Santana dos Santos, T.F., Aquino, A., Silveira Dórea, H., Navickiene, S. 2008 MSPD procedure for determining buprofezin, tetradifon, vinclozolin, and bifenthrin residues in propolis by gas chromatography–mass spectrometry Anal. Bioanal. Chem. 390: 1425-1430.

[2] Horwitz, W., Albert, R. 2006. The Horwitz Ratio (HorRat): a useful index of method performance with respect to precision. J. AOAC. Int. 89: 1095-1109.

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