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Introduction

Propolis is a valuable by-product from bees. Due to its antiseptic properties, it is widely used in pharmaceutical and food industries. Bees collect propolis from the hive surroundings producing a dense mass of polyphenols, terpenic resins and waxes that they use as a general purpose sealant and to prevent the hive from bacterial and fungal attack [1]. Pesticide residues in propolis arise from two main sources, either from environmental contamination due to agricultural practices or through pesticide application in the hive, to prevent parasitic acaroids like *Varroa destructor* or ants.

Varroa sp. is a major hive disease, dropping down hive productivity and hampering the normal development of the colony. Therefore, acaricides are widely employed in apiculture and are common propolis contaminants that are currently controlled, although neither official method, nor regulation is given for this product.

A simple method for organophosphate pesticide residue analysis based on MSPD and SiO₂ column chromatography in Propolis for pharmaceutical use is presented.

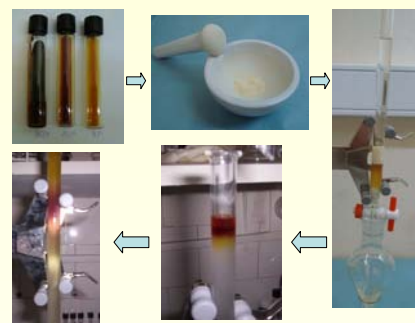
Experimental

1.- MSPD based extraction

- 1 g of 80 % propolis formulation is weighed into a 10 mL volumetric flask and diluted with acetone.
- 1.00 mL of diluted sample is blended in a mortar with 2 g of Al₂(SO₄)₃ to dryness
- The mixture is placed in a (250 mm x 14 mm i.d) glass column packed with 2 g of Florisil.
- Elution is performed with 30 mL of CH₂Cl₂:AcOEt (9:1) mixture.
- Solvent is evaporated to dryness under reduced pressure.

2.- Column chromatography clean-up

- Extract is diluted in 2 mL of CH₂Cl₂
- The mixture is placed in a (250 mm x 14 mm i.d) glass column packed with 7 g of Silica.
- Elution is performed with 30 mL of CH₂Cl₂
- Solvent is evaporated until dryness under reduced pressure.
- 1.00mL of Bromophos-methyl (internal standard) in AcOEt is added and directly analyzed by GC.



The pesticide residues were analyzed by a Gas-Chromatograph Shimadzu GC-17A equipped with a flame photometric detector (FPD).

Column: Mega 68, thickness: 0.25 μm, large: 25 m, d.i: 0.32 mm. Flow 1.7mL/min using He as carrier gas, Injection volume: 1 μL. PTV Injector, Temperature:270°C (25 min).

Oven temperature programme: 150°C (2 min), to 270°C (7 min) at 10°C/min.

Results & Discussion

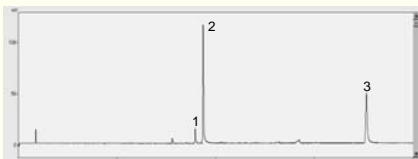


Fig. 1: FPD chromatogram of a real propolis sample. 1= chlorpyrifos, 2=bromophos-methyl (IS), 3=coumaphos

Pesticide	R.T (min)	R.T.T	Volume (mL)	% Rec	RSD
Chlorpyrifos	8,961	0,95747409	25	79,2	43,2
			30	103,9	2,1
			35	96,3	5,8
Ethion	11,989	1,28101293	25	78,0	46,6
			30	100,0	0,1
			35	95,7	4,5
Coumaphos	17,615	1,88214553	25	70,1	9,1
			30	74,1	16,2
			35	101,6	15,2
Bromophos-methyl	9,359	1	35		

Table 1: Retention time, relative retention time, CH₂Cl₂ volume (mL), Recoveries (%) at 0.2 mg/kg and RSD of the selected pesticides

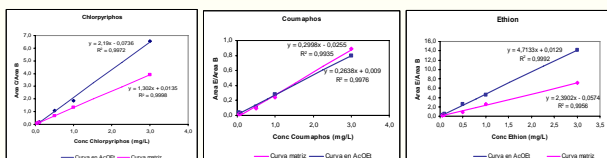


Fig.3: Calibration curves in AcOEt and Matrix-matched calibration.

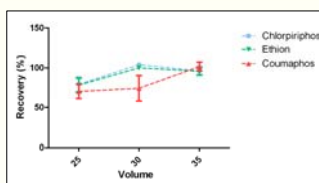


Fig. 2: Effect of the CH₂Cl₂ volume (mL) on the recoveries in the clean-up step.

➤ Different parameters were optimized for method development. 30 mL of CH₂Cl₂ was selected as elution volume in the clean-up step.

➤ Recovery studies showed percentages that ranged from 74.1 to 103.9 with RSD < 16.2 %.

➤ Matrix effect was evaluated and significant differences were found in chlorpyrifos and ethion response for this matrix.

➤ This is the first record of OP pesticide residue analysis in propolis formulations.

Conclusions

This method provides a suitable methodology for the analysis of *Varroa sp.* controllers commonly used in Uruguay such as Coumaphos and other organophosphate pesticides in propolis extracts for pharmaceutical use without interferences.

References

- [1] Santana dos Santos T.F, Aquino A, Silveira Dórea H & Navickiene S, *Anal. Bioanal. Chem.* 2008 390, 1425-1430

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API TER Ltda.
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