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DETERMINATION OF ACRINATHRIN RESIDUES IN HONEY AND BEESWAX

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The Asian bee mite (*Varroa jacobsoni* Oud.) causes variable damage in Hungarian apiaries due to the different and changing conditions. Plastic and wooden strips impregnated with synthetic pyrethroid-type active ingredients show a high efficacy against the mites. These treatments, however, may leave residues in bee products (honey, propolis, wax). After experimental treatment with Gabon PA 92, the levels of active ingredient (acrinathrin) residues were determined in honey and beeswax samples. The analytical results proved that the average concentration of acrinathrin residues was less than 0.01 mg/kg in honey and less than 0.10 mg/kg in beeswax. From the food-hygienic point of view it is favourable that the honey did not become 'contaminated' with acrinathrin during the experimental treatment. The analytical results serve as a basis for the registration of this veterinary product in Hungary.

Key words: Bee acaricide, acrinathrin, pyrethroid, residues, Gabon PA 92, honey, beeswax

The continuous damage done by the Asian bee mite (*Varroa jacobsoni* Oud.) since 1978 made it necessary to hold an international symposium in Prague in 1993 to discuss methods for the control of this parasite. At that symposium different biological, mechanical and chemical tools were presented.

In Hungary, the living conditions of bees, the short distance between apiaries as well as the wandering of bee-keepers make it impossible to eradicate the mite completely. The damage of this parasite varies as a result of the dissimilar and continuously changing conditions. Its presence is, therefore, always presumable and should be considered. At the moment, we cannot protect our colonies either by biological breeding methods (Woyke, 1989; Bienefeld and Pritsch, 1992; Büchler, 1993; Hoffmann, 1993; Rosenkranz et al., 1990) or by applying materials of natural origin (Wallner, 1993). As a complement to other methods,

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the application of synthetic chemicals with different active ingredients is accepted in Hungary (Tóth, 1998).

The prolonged application of these chemicals may lead to the development of resistance (Hillesheim et al., 1996) or cross-resistance (Milani et al., 1995) and to the accumulation of the compounds in beeswax (Wallner, 1997).

Our aim was to find a method providing acceptable protection against the Varroa mite with minimal exposure to chemicals. Therefore, we started experiments with the application of Gabon PA 92 wooden strips. This veterinary product proved to be effective. Our earlier trials (lasting several years) showed that a single treatment performed in autumn was sufficient for maintaining the bee colonies in healthy condition and keeping the number of mites on an acceptably low level.

Consumers are increasingly aware of the hazard posed by the chemical contamination of food. With this in view, an experimental treatment was carried out to determine the levels of acrinathrin residues in apicultural products.

For the first time in August 1994, a comparative experimental treatment was performed in the apiary of the Institute for Small Animal Research to test Gabon PA 92 strips. In this experiment the efficiency of Gabon PA 92 was compared with that of Apistan (with fluvalinate active ingredient) and a home-made wooden plate impregnated with Klartan (also containing fluvalinate). The experiment involved laboratory and open field tests of both brood and adult bees (Szalai et al., 1998).

Materials and methods

Gabon PA 92 strips contain acrinathrin in a quantity of 1.2–1.7 mg/strip. Figure 1 shows the structure of the active ingredient, which belongs to the group of synthetic pyrethroids.

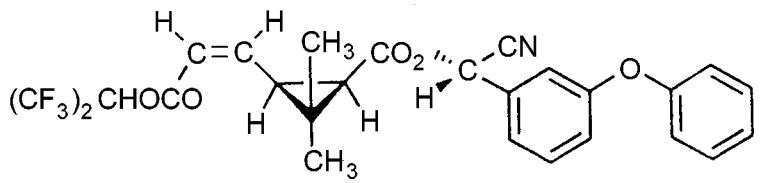

Fig. 1. Structure of acrinathrin. Active ingredient: (S)-α-cyano-3-phenoxybenzyl (Z)-(1R,3S)-2,2-dimethyl-3-[2-(2,2,2-trifluoro-1-trifluoromethylethoxycarbonyl) vinyl] cyclopropanecarboxylate (IUPAC)

After a single treatment in 1995 and 1996 respectively, in the spring of 1997 the following experimental treatment was performed. Five bee colonies were treated by hanging 2 GABON PA 92 strips into every beehive for 25 days.

After this treatment the amount of active ingredient residue in honey and beeswax samples taken from beehives treated with GABON PA 92 was determined.

Two weeks after removing the strips from the treated beehives (at the time of honey extraction) honey and wax samples were taken. Untreated (control) samples were also taken at the same time. To avoid degradation of the active ingredient, the samples were stored at –18 °C.

The appearance of the lipophilic active ingredient was expected first of all in the beeswax. Therefore, honey and beeswax samples were analysed for residue content separately, according to the sample preparation and analytical method presented in Figs 2 and 3. The analysis consisted of the following stages: sampling, extraction, clean-up, gas chromatographic determination with external standardisation.

Sample preparation was based on the method introduced by Vesely et al. (1995). The acrinathrin residue was determined by gas chromatography with electron capture detection. The parameters of gas chromatography are shown in Table 1.

Parallel to the treated samples we examined control honey and beeswax samples from an untreated beehive and samples spiked with active ingredient. Acrinathrin was added to honey and beeswax in 0.05–5.0 mg/kg amounts, and subsequently the recovery rates (%) were determined. The mean recovery rate was 96.7% for honey samples and 88.3% for beeswax samples (Table 2).

Results

The results are summarised in Table 3. The data represent the means of the results of two parallel measurements each.

Blind studies of the chemicals involved did not show any cross-contamination. Acrinathrin residues were not detected in the extracts of control honey and wax samples, which indicates residue levels of < 0.01 mg/kg for honey and < 0.10 mg/kg for beeswax.

Discussion

The analytical results proved that the residue levels of active ingredient were less than 0.01 mg/kg in honey and less than 0.10 mg/kg in beeswax.

From the food-hygienic point of view it is favourable that acrinathrin residues were not detectable in any of the processed honey and wax samples. During the experimental treatment the honey did not become ‘contaminated’ with acrinathrin.

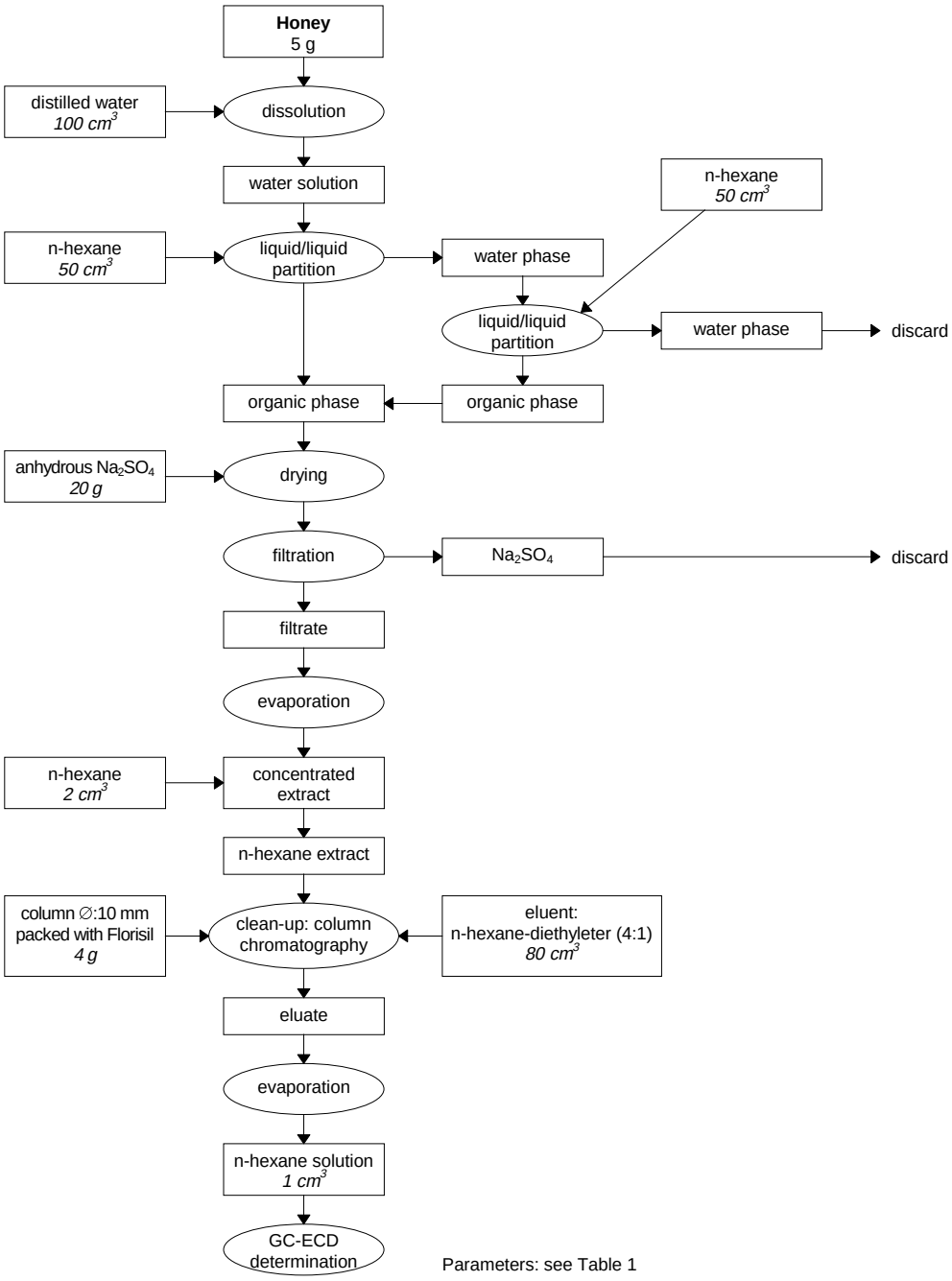

Fig. 2. Analytical method for the determination of acrinathrin residues in honey

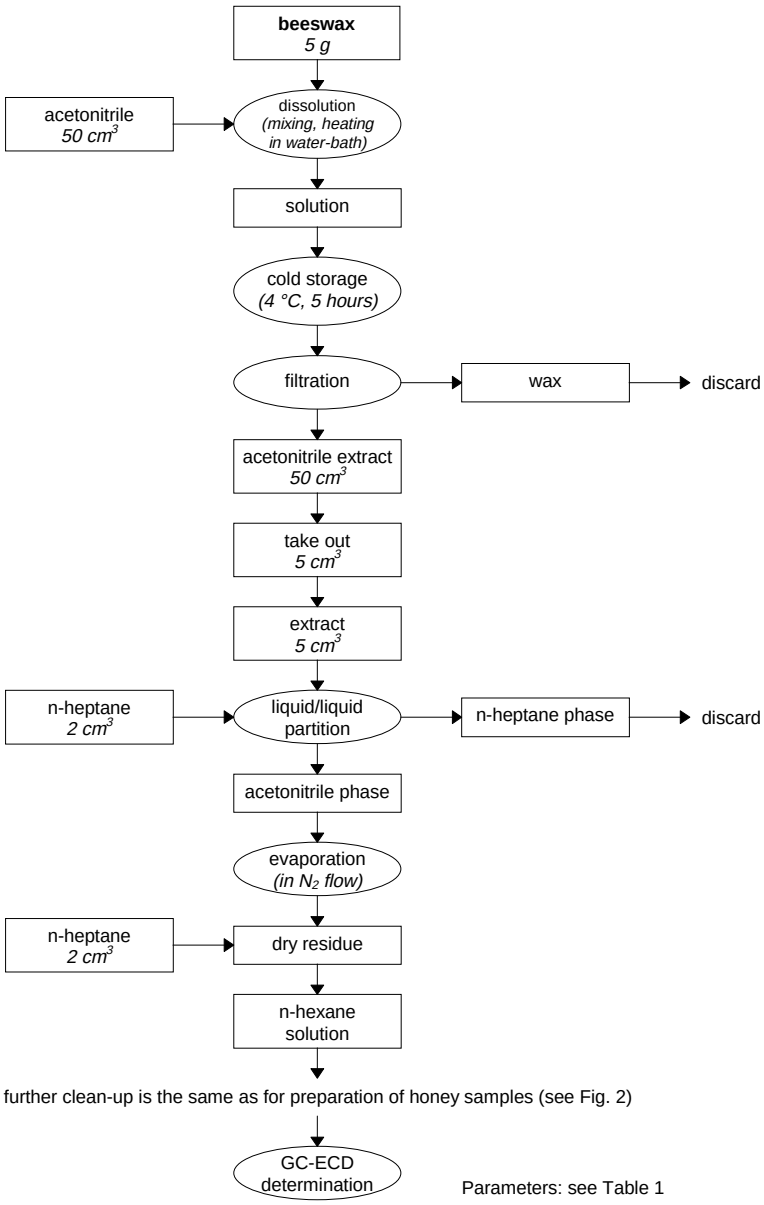

Fig. 3. Analytical method for the determination of acrinathrin residues in beeswax

Table 1

Parameters of the gas chromatographic determination

	I. For honey extracts		II. For beeswax extracts
Instrument	Chrompack 9000		Perkin Elmer Sigma 3B
Detector	ECD (Ni 63)		ECD (Ni 63)
Column	25 m × 0.32 mm i.d.∅ CP Sil PAH CB d_f = 0.12 μm	25 m × 0.32 mm i.d.∅ HP Ultra 1 d_f = 0.52 μm	1 m × 2 mm i.d. ∅ glass 2% OV 101/ GasChrom Q 100–120 mesh
Carrier gas	nitrogen of high purity 3 cm^3/min		nitrogen of high purity 45 cm^3/min
Make-up gas	nitrogen of high purity 30 cm^3/min		nitrogen of high purity 30 cm^3/min
Split proportion	5:1	5:1	–
Temperatures			
injector	240 °C	240 °C	240 °C
column	220 °C	230 °C	220 °C
detector	280 °C	300 °C	280 °C
Acrinathrin retention time	3.89 min	10.34 min	3.00 min
Detection limit:			
from honey	0.01 mg/kg	0.01 mg/kg	
from beeswax	0.10 mg/kg		0.10 mg/kg

Table 2

Recovery rates in the course of determination of acrinathrin residues

Sample	Fortification level (mg/kg)	Mean recovery (%)
Control honey	0.05–0.20	96.7 (n = 5)
Control beeswax	1.00–5.00	88.3 (n = 4)

The results of this residue determination trial serve as a basis for the registration and marketing authorisation of the veterinary product GABON PA 92 in Hungary. In the future, the investigations will be extended to residue tests of acrinathrin in samples taken from different locations and at different intervals after treatment. For health protection purposes and to maintain the exportability of Hungarian honey, further tests will be performed with acrinathrin and other active ingredients of bee acaricides (fluvalinate, flumethrin, amitraz).

Table 3

Residue content of honey and beeswax samples taken from beehives treated with GABON PA 92 strips against Varroa mites

Sample (no. of bee colony)		Acrinathrin residue content (mg/kg)
Honey	22	< 0.01
	34	< 0.01
	46	< 0.01
	56	< 0.01
	60	< 0.01
Beeswax	22	< 0.10
	34	< 0.10
	46	< 0.10
	56	< 0.10
	60	< 0.10

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