DETERMINATION OF PIPERONYL BUTOXIDE IN HONEY BEES BY GAS CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY

DETERMINAREA PIPERONIL BUTOXIDULUI DIN ALBINE PRIN GAZ-CROMATOGRAFIE CUPLATĂ CU SPECTROMETRIE DE MASĂ

Cristina ȚOCA[1)], Daniela NICA[1)],
Adriana PANCHIOSU[1)], Angelica GHEORGHE[1)]

ABSTRACT | REZUMAT

Piperonyl butoxide (PBO) is a sintetic synergist which itself is not harmfull for insects, but in combination with pesticides like pyretroids or carbamates increase their effectiveness. Usualy, PBO is found in particular in products used inside homes like mosquito, flies, and also flea and tick treatments for pets. PBO is not designed to kill insects by itself. PBO is practically non-toxic to birds, mammals and bees by itself (DL50 ≥ 25 µg/bee) and moderately toxic to fish.

This paper presents a method for the determination of PBO in honey bees samples due to a high number of intoxication registrared with pesticides.

In the toxicology lab was developed a method for identification of PBO in honey bees by gas chromatography coupled with tandem mass spectrometry.

Sample preparation procedure consists in weighing a small amount of honey bees sample, homogenization with dry sodium sulphat, extraction with ethyl acetate under strong ultra-sonication, dispersive solid phase extraction with C18 and PSA, evaporation under nitrogen stream and redissolution with a mixture of cyclohexane-acetone. The identification of the PBO it's made on the basis of the spectrum and the retention time. In the last few years were registrated several cases of intoxication in honey bees with pyrethroids like cypermethrin, tefluthrin, deltamethrin, tetramethrin together with PBO. This PBO in honey bees samples, together with a pesticide (usually piretroid), can be an indication that their death was caused intentionally using a commercial/ inside home product intended to control domestic insects.

Key words: honey bees, piperonyl butoxide, gas chromatography tandem mass spectrometry

Piperonil butoxidul (PBO) este un sinergist sintetic care nu este toxic pentru insecte, dar combinat cu pesticide ca piretroizi sau carbamați, le potențează efectul insecticid. De obicei, acesta se găsește, în special, în produsele destinate combaterii insectelor casnice cum sunt țânțarii, mușteleetc. precum și în produsele utilizate pentru efectuarea tratamentelor specifice la animalele de companie în controlul puricilor și căpușelor. PBO nu omoară insectele. PBO este practic netoxic pentru păsări, mamifere și albine (Dl50 ≥ 25 µg/ albină) și moderat toxic pentru pești. Lucrarea prezintă o metodă de determinare a PBO alături de pesticide din probe de albine, datorită înregistrării unui număr mare de intoxicații la aceste insecte.

În cadrul laboratorului de toxicologie a fost elaborată o metodă de determinare a piperonil butoxidului alături de pesticide din probe de albine, bazată pe gaz-cromatografie cuplată cu spectrometrie de masă.

Metoda folosită pentru pregătirea probelor constă în extracția prin ultrasonare a compușilor cu acetat de etil în prezența sulfatului de sodiu anhidru, purificarea cu C18 și PSA, concentrare și reluarea reziduului în amestec de ciclohexan-acetonă. Identificarea PBO și a pesticidelor se face pe baza spectrului de masă și a timpului de retenție. În ultimii ani au fost înregistrate mai multe cazuri de intoxicații la albine cu piretroizi cum ar fi cipermetrin, teflutrin, deltametrin, tetrametrin, alături de care s-a indentificat PBO. Prezența PBO în probe de albine, alături de un pesticid (de obicei piretroid) poate fi un indiciu că moartea acestora a fost provocată intenționat, utilizându-se un produs comercial destinat combaterii insectelor casnice.

Cuvinte cheie: albine, piperonil butoxid, gaz-cromatografie cuplată cu spectrometrie de masă

Piperonyl butoxide (PBO) (Fig. 1, 2), is a sintetic synergist which itself is not harmfull for insects, but in combination with pesticides like pyretroids or carbamates increase their effectiveness. Usualy, PBO is found in particular in products used inside homes like mosquito, flies, and also flea and tick treatments for pets. PBO is not designed to kill insects by itself. Insects have enzymes in their bodies that break down some insecticides. PBO stops some of these enzymes

1) Institute for Diagnosis and Animal Health, Romania
E-mail: toca.cristina@idah.ro

and allows insecticides more time to work. PBO is quickly broken down in the presence of sunlight, with a half-life of 8.4 hours in water and 3.4 hours in air. Without sunlight, the half-life increased to 14 days. PBO is practically non-toxic to birds, mammals and bees by itself (DL 50 ≥ 25 µg/bee) and moderately toxic to fish.

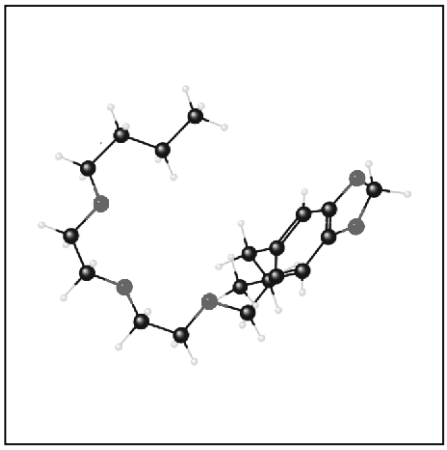

Fig. 1. 3D Structure of PBO

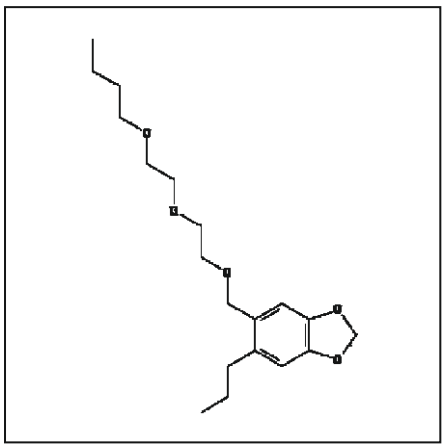

Fig. 2. 2D Structure of PBO

PBO acts as an insecticide synergist by inhibiting the natural defense mechanisms of the insect, the most important of which is the mixed-function oxidase system, (MFOs) also known as the cytochrome P-450 system. The MFO system is the primary route of detoxification in insects, and causes the oxidative breakdown of insecticides such as pyrethrins and the synthetic pyrethroids - thus when PBO is added, higher insecticide levels remain in the insect to exercise their lethal effect. An important consequence of this property is that, by enhancing the activity of a given insecticide, less may be used to achieve the same result. PBO is found to be an efficacious, low-potency, neutral antagonist of G-protein-coupled CB1 receptors.

It has been used in pesticide products since the 1950s, when it was first registered in the United States.

There are more than 2,500 pesticide products that contain the active ingredient PBO. These include foggers, dusts, and sprays. Some of these products may be used inside and outside of homes. PBO is used on agricultural crops and livestock. Other uses include mosquito to control programs and flea and tick treatments for pets. Early studies found that PBO greatly improved how well pyrethrins kill houseflies. PBO itself did not kill the flies. The combination of both allowed more control with smaller amounts of pyrethrins.

This paper presents a method for the determination of PBO and pesticides in honey bees samples due to a number of intoxication registrared with pesticides.

MATERIALS AND METHOD

All compounds were obtained from Sigma-Aldrich with purity higher or equal than 97%. Stock standard solution of each compound at 1000 mg/l were prepared in cyclohexane-acetone and stored at -20°C. Working standard solutions of a mixture PBO-pesticides were prepared appropriately by diluting standard stock solutions with cyclohexane-acetone and stored at -20°C was stable at least 6 months. For purification were used C18 for column chromatograpy (Fluka) and PSA bonded silica (Supelco).

Analysis was carried out with a GC-MS system, Trace GC coupled to an ion trap mass spectrometer Polaris Q with mass spectrum library (The NIST Mass Spectral Search Program for NIST/EPA/NIH Mass Spectral Library, 2.2. version/10.06.2014) made by Thermo Scientific, USA; data acquisition and processing was performed using Xcalibur software. (Fig. 3)

Fig. 3. GC-MS system, Trace GC coupled to an ion trap mass spectrometer Polaris Q with mass spectrum library

Chromatographic separation was performed on a 30m x 0.25mm ID, 0.25 µm film thickness TraceGOLD TG-5SilMS capillary column.Hellium (purity 99.999%) was used as a carrier gas at a constant flow of 1 ml/min. Initial oven temperature was set at 100°C for 1 min., followed by a linear ramp to 150°C at a rate of 25°C/min. Subsequently, the temperature was raised to 280°C at a rate of 6°C, hold 12 min. A split-spitless injector set at 280°C was always used and injections were performed in splitless mode. Injection volume was 2 µl. A solvent delay of 10 min. was applied. Transfer line temperature was set at 280°C and the source at 250°C. The mass operated in EI mode, an electron energy of 70 eV and a filament emission current of 250 µA. The scan mode was used between *m/z* 50 and 550. The sample preparation included weighing of 16 bees in 50 ml Falcon tube, homogenising with dry sodium sulphat using a glass rod, extracting with ethyl acetate under strong ultra-sonication, dispersive solid phase extraction with C18 and PSA and evaporating under nitrogen stream and re-dissolution with a mixture of cyclohexane-acetone.

RESULTS AND DISCUSSIONS

PBO and pesticides were identificated by mass spectrum and the retention time.

In the figures below (Fig. 4, 5, 6) are ilustrated the chromatograms that indicates a case of intoxication with pyrethroids (cypermetrin, tetrametrin) in honey bees; in the same sample it was identify PBO. The characteristic mass fragment ions used for qualitative analisys and the retension time (RT) are given in Table 1

Table 1

The characteristic mass fragment ions used for qualitative analisys and the retension time (RT)

Compound	RT (min.)	Ion for identification (*m/z*)
Cypermethrin	21.25	165, 209, 181
Tetramethrin	25.53	165, 165, 107
PBO	20.33	176, 177

CONCLUSIONS

In this paper, a screening method developed for the identification of pesticide in honey bees, but which can be used to identify a lot of compounds such as PBO. This method needs a small quantity of sample and an easy preparation procedure due to the power of GC MS technique. The time, labor and volume of reagents consumption are minimized.

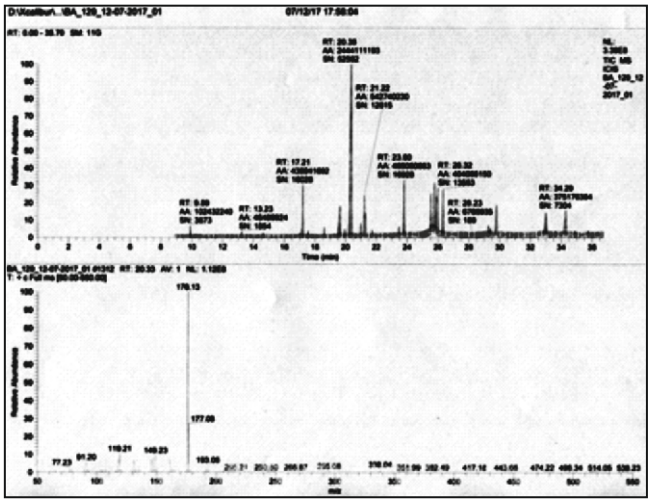

Fig. 4. The total ion chromatogram (TIC) and mass spectrum of PBO identified in honey bees

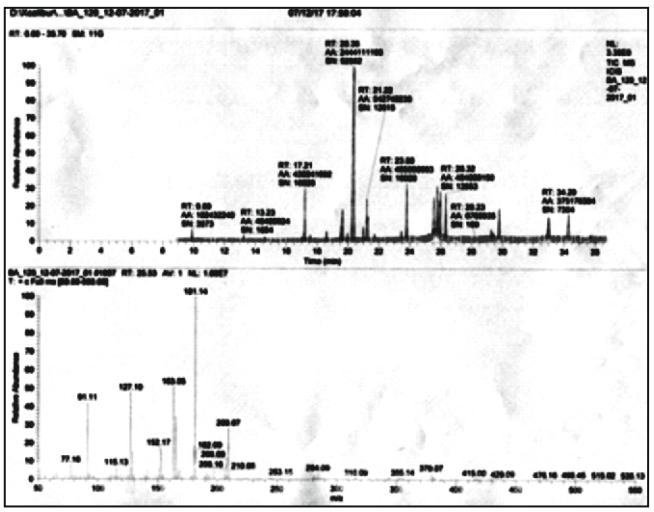

Fig. 5. The total ion chromatogram (TIC) and mass spectrum of cypermethrin identified in honey bees

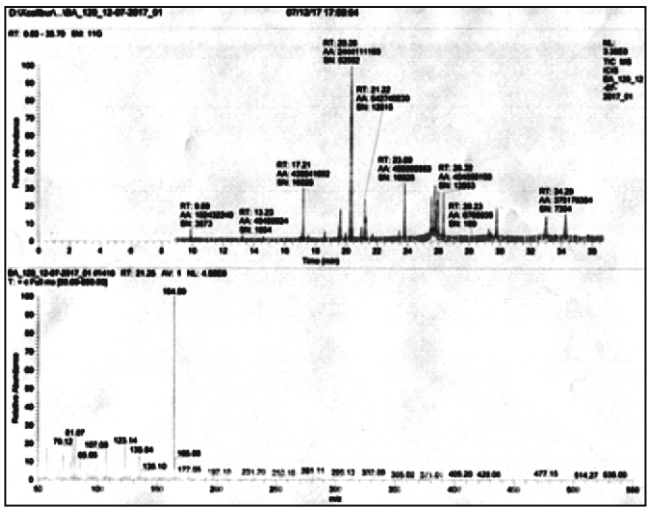

Fig. 6. The total ion chromatogram (TIC) and mass spectrum of tetramethrin identified in honey bees

In addition, identification of PBO in honey bees samples together with a pesticide (usually piretroid) can be an indication that the death of the insects was caused intentionally, using a commercial/ inside home product intended to control domestic insects.

PBO is regulated in the United States and some other countries as a pesticide, even though PBO does not have this property. The United States Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the law that gives United States EPA its authority to regulate pesticides, includes certain synergists in its definition of a "pesticide" and is thus subject to the same approval and registration as products that kill pests, like the insecticides with which PBO is formulated. Pesticide registration is the process through which is examinated the ingredients of a pesticide, where and how the pesticide is used (e.g., whole room fogger, crack-and-crevice, etc.), and the specific use pattern (amount and frequency of its use). Pesticides are also evaluates in the way to ensure that it will not have unreasonable adverse effects on humans, the environment and non-target species. The pesticides must register before they may be sold or distributed in the market. Registration is required for the pesticide itself, as well as for all products containing it.

The World Health Organization recognizes the public health value of PBO when used in conjunction with the synthetic pyrethroids deltamethrin or permethrin used in mosquito nets.

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