Microquantities of Pesticides in Bees and Bee Products: Modern Analytical Techniques

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Abstract: A large number of pesticides are currently used in agriculture. They are represented by groups of organochlorine and organophosphorus pesticides, synthetic pyrethroids, amides and carbamates and some others. The contamination of products with organochlorine pesticides, which were banned decades ago, but due to their high persistence in the environment, continue to circulate in biocenoses, continues to be relevant. Modern analytical equipment makes it possible to optimize methods for analyzing microquantities of pesticides, covering the widest possible range of substances. At the same time, it is necessary to strive to reduce labor costs and the consumption of organic solvents, for which it is necessary to develop a unified methodology.

1 INTRODUCTION

Currently, a large number of pesticides with different physicochemical properties are used. Organochlorine pesticides(OCPs), which were banned decades ago, continue to persist in biocenoses and accumulate in the upper links of the food chain, they are extremely stable, and still retain toxicological significance not so much for bees, but for humans - consumers of beekeeping products. Organophosphorus(OPPs) compounds and synthetic pyrethroids(SPs) are less stable and are quickly destroyed in biocenoses, but they are highly toxic drugs for bees, as a result of which they often cause poisoning of bees of varying degrees, up to the complete death of the bee colony. Neonicotinoids can accumulate both in nectar, guttation fluid and pollen of plants, and remain in the soil for several seasons, and penetrating into the biological environment of plants planted on contaminated soil, causing poisoning of bees. The wide range of pesticides used at the present stage requires sample preparation and an analysis method separately for each group of drugs. This leads to high consumption of organic solvents, high labor intensity, and requires several analytical instruments. Therefore, researchers are faced with the task of developing a unified methodology that makes it possible to determine the maximum range of substances in the maximum number of matrices of bee products. This article presents the most promising methods for the analysis of a wide range of pesticides in various matrices.

2 MATERIALS AND METHODS

The research material included publications related to ecology, the use of pesticides and methods of their detection in bees and bee products.

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The research method was to search for publications on the topic of accumulation of pesticides in bees and bee products. The search was carried out using the Google Scholar, Cyberleninka, Springer, Scopus databases, as well as printed publications. Preference was given to publications for the period from 2013 to 2023; if there was a lack of information, older sources were used. The search was carried out using the keywords organochlorine pesticides, organophosphorus pesticides, synthetic pyrethroids, amides, carbamates, bee products, contamination

3 RESULTS AND DISCUSSION

The most dangerous pesticides for bees have been identified, which are most often used to treat sunflower and corn in Spain. Pesticide residues were analyzed by GC-MS and HPLC-MS. There were separate methods for fipronil and imidacloprid. For analysis of OCPs, OPPs and pyrethroids, a 4-gram aliquot of frozen pollen was mixed with sodium sulfate, ground in a mortar, and extracted with 10 mL of acetonitrile by shaking. The liquid phase was separated by centrifugation and 100 ml of water was added before elution through a 500 mg ODS cartridge. The stationary phase was washed with 10 mL of 20:80 (v/v) methanol:water and the analytes were eluted with 3 mL of acetone. The acetone was evaporated on a rotary evaporator, the residue was dissolved in 1 ml of acetone and filtered through a PTFE filter with a pore size of 0.45 μm and analyzed using GC-MS. For the analysis of carbamates, azole fungicides and other thermally unstable substances, another 4 gram aliquot of frozen pollen was mixed with sodium sulfate and extracted twice with 20 ml of acetone, diethyl ether and methylene chloride. The liquid phases were combined and evaporated to dryness in a rotary evaporator (Bernal J. et al., 2010).

A honey sample weighing 10 grams was dissolved in 100 ml of 4% aqueous sodium sulfate, after which it was extracted with 40, 30, 30 ml of ethyl acetate, the extract was centrifuged at 3000 rpm for 5 minutes. The organic phase was filtered through a 5 cm layer of anhydrous sodium sulfate. Then concentrated to 5 ml for analysis. Analyzed by GLC, OCPs, OPPs and carbamates were determined (Khan M. S. et al., 2004). A review has been published on methods for detecting pesticides in bees and bee products (Bargańska Ż., Namieśnik J., 2010).

A 10 gram sample of honey was weighed in an Erlenmeyer flask, 5 ml of water was added to the sample, and stirred until dissolved. Then 50 ml of a solution of hexane and acetone (60:40 by volume) was added and extracted with shaking for 20 minutes. The organic phase was then separated by centrifugation at 2500 g for 10 minutes. The extraction was then repeated using 40 ml of hexane:acetone, after which the combined extracts were evaporated on a rotary evaporator under reduced pressure at 65 degrees and the sample was dried under a stream of pure nitrogen. The residue was dissolved in 5 ml of acetone and filtered through a PTFE filter with a pore size of 0.5 μm. For SPE purification, Florisil cartridges were used, which were pretreated with approximately 5 ml of a mixture of equal amounts of ethyl acetate and hexane. Then, without allowing the cartridge to dry out, the sample extract was added there and eluted first with 5 ml of a mixture of methylene chloride and hexane (80:20 by volume), then with 5 ml of a mixture of hexane and acetone (60:40 by volume). The mixture was evaporated, the residue was dissolved in 1 ml of acetone, and analyzed by GLC-ECD. OPPs, OCPs, pyrethroids, and nitrogen-containing pesticides are determined (Rissato S. R. et al., 2004).

A method has been proposed for analyzing honey, bees and pollen for the content of pesticides of the OCP, OPP groups, synthetic pyrethroids, amides, and carbamates. Honey was weighed in a 50 ml centrifuge tube, 10 ml of water was added, and shaken until dissolved. Then 10 ml of acetonitrile and a mixture of salts consisting of 4 g of anhydrous magnesium sulfate, 1 g of sodium chloride, 1 gram of sodium citrate dihydrate, 0.5 g of disodium citrate sesquihydrate were added. A solution of isotope-labeled pesticides was added as an internal standard. Shake first by hand, then vortex for 1 minute, then centrifuge for 2 minutes at 5000 g. A 6 ml aliquot was then placed into a 15 ml SPE tube, the tubes were shaken by hand, then vortexed for 10 seconds, and centrifuged for 2 minutes at 5000 g. An aliquot of 4 ml was taken into a glass centrifuge tube and evaporated to 50 μl and analyzed. When analyzing bees, a sample of 10 grams is crushed, after which 5 grams are weighed in a 50-ml centrifuge tube, 10 ml of acetonitrile, 3 ml of water, 3 ml of hexane, a mixture of salts, and an internal standard were added. Shake first by hand, then vortex for 1 minute, then centrifuge for 2 minutes at 5000 g. Then 6 ml of acetonitrile fraction (under hexane) was transferred into C18 SPE tubes, shaken manually, then vortexed for 10 seconds, centrifuged for 2 minutes at 5000 g. An aliquot of 4 ml was taken into a glass centrifuge tube and evaporated to 50 μl and analyzed. For analysis, pollen was weighed in a 50-ml test tube, 10 ml of acetonitrile, 8 ml of water, 3 ml of hexane, and a mixture of salts were added. Then the analysis proceeds as for bees (Wiest L. et al., 2011).

The LLE method was used to determine OPPs, OCPs, SPs, and cyclodiene pesticides in honey. Modified methods were used for the analysis of OCPs, cyclodienes and SP (Chawla R. P., Goyal N. P., 1988). A 50 gram sample of honey was weighed in a 250 ml conical flask, 100 ml of water and 100 ml of methanol were added. Shake for 3 hours and filter through a paper filter. For purification, the extract was transferred to a 500 ml separating funnel and 100 ml of hexane was added. Shake for 15 seconds, then separate the organic layer and dry over anhydrous sodium sulfate. The extraction was repeated. Concentrated to 1-2 ml by evaporation under vacuum, then transferred to a glass column containing florisil and silica gel in a 1:1 ratio. Elute with hexane to a final volume of 30 ml. For the analysis of OPPs, methods were modified (Sharma D. C., Kashyap N. P., 2002, Khan M. S. et al., 2004). A 10 gram sample of honey was dissolved in 100 ml of 4% aqueous sodium sulfate solution in a 250 ml conical flask, shaken for 1 hour, filtered through a paper filter. 50 ml of the solution was transferred to a 350 ml separating funnel and extracted 4 times with 50 ml of hexane. Each hexane fraction was centrifuged for 5 minutes at 5000g. The extract was then dried with 15 grams of anhydrous sodium sulfate and concentrated to 1-2 ml by evaporation under vacuum at 35 degrees. transferred to a glass column containing Florisil and silica gel in a 1:1 ratio. Eluted with hexane. It was evaporated, the dry residue was dissolved in 5 ml of hexane, and analyzed (apparently, the same was done for OCPs and pyrethroids) by GLC with ECD (Choudhary A., Sharma D. C., 2008).

A large number of pesticides have been identified in honey, fruit juice and wine using GLC-MS and HPLC-MS-MS methods. 15 grams of sample was weighed into a 250 ml flask with an accuracy of 0.01 g. Add 30 ml of water and shake for 15 minutes in a water bath at 40 degrees. Add 10 ml of acetone and transfer the liquid to a 250 ml separating funnel. The flask was washed with 40 ml of methylene chloride in several stages and added to the liquid in a separating funnel. Shaking too long results in an emulsion, while not shaking enough results in incomplete extraction of the pesticides. Tests have shown that it is optimal to shake the funnel about 8 times. Filter the lower phase through anhydrous sodium sulfate, add 5 ml of acetone and 40 ml of methylene chloride into a separating funnel and repeat the extraction twice more. Concentrate the extract to approximately 1 ml. To clean a cartridge with graphitized soot, pour anhydrous sodium sulfate in a layer of 2 centimeters, attach the cartridge to an aminopropyl cartridge, condition by passing 4 ml of a mixture of acetonitrile:toluene (3:1). Elute the pesticides with 25 ml of the same mixture. Concentrate the eluate to 0.5 ml under vacuum at 40 degrees. The pesticides are then extracted with hexane or methanol (conditions and a list of 450 pesticides are described in the article) and analyzed (Pang G. F. et al., 2006).

10-gram samples of honey in glasses were dissolved in 30 ml of water and 20 ml of saturated sodium chloride solution. Homogenize for 5 minutes at a temperature of 50 degrees in a water bath and transfer to 150 ml centrifuge tubes. After adding 40 ml of methylene chloride, the tubes were shaken vigorously for approximately 5 minutes and centrifuged at 5000 rpm for 10 minutes. The organic layer was dried with anhydrous sodium sulfate and transferred to a 250 ml round bottom flask. The aqueous layer was extracted two more times with 40 ml of methylene chloride, 1 ml of isooctane was added and evaporated to 2 ml, the final volume was 1 ml (Balayiannis G., Balayiannis P., 2008).

10-gram samples of honey were dissolved in 30% aqueous methanol (10 ml), standard solutions of pesticides in ethyl acetate were added, and 0.5 ml of ethyl acetate was added to uncontaminated honey. A column containing 1 gram of C18 was conditioned first with 3 ml of acetonitrile, then with 5 ml of water. The honey solution was passed through a column and collected in 10 ml test tubes. Pesticides are retained in the solid phase, from where they are eluted twice with 5 ml of a mixture of equal amounts of hexane and ethyl acetate. It was dried over anhydrous sodium sulfate and analyzed (Albero B., Sánchez-Brunete C., Tadeo J. L., 2004).

A screening method has been developed for the determination of OPPs in honey and bees using HPLC-MS. 0.5 grams of bees in a mortar were carefully mixed with 1 gram of C18 for 5 minutes using a pestle. The mixture was placed in a 9*100 mm glass chromatographic column containing 0.5 grams of florisil, topped with silanized glass fiber. Pesticides were eluted with 10 ml of a mixture of methylene chloride and methanol (85:15) and concentrated to 1 ml. Filtered through a 0.22 μm PTFE filter and analyzed. 5 grams of honey were mixed with 50 ml of water and shaken for 10 minutes. The solid phase was conditioned with 10 ml of methanol and 10 ml of water using a vacuum pump, without allowing the solid phase to dry out, the sample solution was passed through it, and the retained pesticides were eluted with 10 ml of ethyl acetate, 4 ml of methanol and 1 ml of dichloromethane. The extract was evaporated to

1 ml and analyzed by HPLC (13. Fernández M., Picó Y., Manes J., 2002).

Before analysis, Florisil was activated by heating for 2 days at 130 degrees. 10 grams of Florisil was filled with a column and 2 grams of anhydrous sodium sulfate was added. For conditioning, 50 ml of a mixture of hexane: methylene chloride (1:1) was passed through the column and then 1 gram of honey was added , dissolved in 2 ml of methanol. Pesticides were eluted with a mixture of hexane and methylene chloride (1:1) in an amount of 30 ml. The mixture was evaporated, after which the residue was dissolved and analysed (Das Y. K., Kaya S., 2009).

Statistics of bee poisoning by pesticides by group in the UK are provided (Fletcher M., Barnett L., 2003).

A 2 gram sample of beebread was weighed in a 50 ml polypropylene centrifuge tube, 5 ml of water, 5 ml of heptane and 10 ml of acetonitrile containing 2% triethylamine were added. The internal standard, acetate buffer (1.5 grams sodium acetate and 6 grams magnesium sulfate) was added and immediately vortexed for 10 seconds by hand to prevent clumping of the magnesium sulfate and vortexed for 20 seconds. The mixture was centrifuged for 2 minutes at 5000 g and 8 ml of the supernatant (acetonitrile layer) was collected. Kept in a plastic tube for 15 hours at -18 degrees, 6 ml of the extract was taken into a centrifuge tube containing 150 mg of PSA and 900 mg of magnesium sulfate, mixed for 10 seconds on a vortex. The extract was centrifuged again for 2 minutes at 5000 g, 4 ml of the supernatant was taken, placed in a glass tube, evaporated at 40 degrees, dissolved in 40 µl of methanol and added 160 µl of water for UHPLC-MS analysis (Giroud B. et al., 2013).

Wax and pollen samples in Spain were analyzed for pesticide content according to the method described in (Orantes-Bermejo F. J. et al., 2010, Bogdanov S., Kilchenmann V., Bütikofer U., 2003).

1 gram of wax was weighed in a centrifuge tube, 10 ml of hexane was added and capped, which was wrapped in Parafilm to prevent opening and evaporation. Extracted in an ultrasonic bath for 45 minutes, shaking manually 2-3 times. Eventually all the wax dissolved. Cooled for 1.5 hours in the freezer, then centrifuged with cooling to -5 degrees at 10,000 g for 15 minutes. The supernatant was decanted into another centrifuge tube and cooled in the freezer for 4 hours. Centrifuged with cooling to -5 degrees at 10,000 g for 15 minutes. The supernatant was poured into a glass tube, closed and heated to room temperature. The column was filled with hexane, 0.1 grams of sodium sulfate and 1.5 grams of Florisil were added. Without allowing it to dry, 5 ml of extract was placed in the column, washed twice with 10 ml of hexane, and the pesticides were eluted with a mixture of hexane and acetone in equal amounts, twice with 10 ml of the mixture. It was evaporated at 40 degrees to a dry residue, dissolved in 2 ml of isooctane, cooled for 2 hours in the freezer and analyzed on a gas chromatograph (Bogdanov S., Kilchenmann V., Bütikofer U., 2003).

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5 CONCLUSIONS

Thus, the laboratory's access to modern analytical equipment makes it possible to maximally expand the range of pesticides being determined, but also to use one method for different matrices. This makes it possible to reduce the complexity of analyzes..

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