Present state and perspectives in analytical methods for pesticide residues analysis in bee pollen: an overview

Mária Rusnáková, Ján Hrouzek & Svetlana Hrouzková

View supplementary material

Published online: 19 Dec 2022.

Submit your article to this journal

Article views: 52

View related articles

View Crossmark data

IBRA
INTERNATIONAL BEE
RESEARCH ASSOCIATION

Taylor & Francis
Taylor & Francis Group

Check for updates

REVIEW ARTICLE

Present state and perspectives in analytical methods for pesticide residues analysis in bee pollen: an overview

Mária Rusnáková, Ján Hrouzek and Svetlana Hrouzková* ORCID

Faculty of Chemical and Food Technology, Institute of Analytical Chemistry, Slovak University of Technology in Bratislava, Bratislava, Slovak Republic

ABSTRACT

Pollen is a set of pollen grains with a microscopic structure derived from plants. In addition to being the main source of nutrition for bees, pollen has also recently become an important part of the food, cosmetic, and pharmaceutical industries. Pollen is gaining attention as a functional food as well as an important sample that has the potential to bioindicate the presence of contaminants in the environment, enabling monitoring of large areas due to the long distances traveled by bees. Pollen matrix is complex and complicated for analysis, therefore, its influence on analytes in samples must be considered. High yields of pesticide residues can be ensured by selecting a suitable sample preparation method, which includes extraction of analytes and purification of extract. A quick, easy, cheap, effective, rugged, and safe method (QuEChERS), which is the most applicable method for the determination of pesticide residues in bee pollen samples, includes an extraction step using extraction salts ($MgSO_4$, NaCl) whose function is to separate the aqueous phase from the organic phase. The next step of QuEChERS is sample purification by using dispersive solid phase extraction (dSPE). The dSPE method combines various sorbents depending on the type of analytes and the effects of the observed matrix. Other techniques used to extract pesticide residues from bee pollen are ultrasonic-assisted extraction, solid phase matrix dispersion, and the mini-salt-liquid extraction technique (SALLE). Gas chromatography (GC) or liquid chromatography (LC) is most commonly used in combination with mass spectrometry (MS/(MS)) employing various mass analysers to determine pesticides.

Introduction

Bee pollen has a chemically and biologically diverse structure that depends on many factors, such as the type of plant from which it comes, climatic conditions, geography, and so forth. Thanks to the nutritional value of pollen, it is nowadays considered a functional food in the food industry. Pollen contains proteins, essential amino acids, carbohydrates, lipids, vitamins, and minerals. In addition to its uses in the food industry, it also has important applications in the cosmetic and pharmaceutical industries, as well as in medicine. Potential bee pollen contamination needs to be controlled. There are many reasons for the need to control levels of pollen contamination. The prime reason is that pollen is used for human consumption. Second, pesticide residues can be concentrated in pollen and therefore pollen can serve as a bioindicator of environmental contamination. Due to the high care of society for a fast and sensitive way to indicate the contamination, this kind of analysis obtains high importance.

One of the most commonly used sample preparation techniques is QuEChERS, which means quick, easy, cheap, effective, rugged, and safe. This technique relies on the extraction of analytes from the sample into an organic solvent, usually acetonitrile. It generally involves an extraction step using extraction salts such as $MgSO_4$ and NaCl to aid the drying and salting-out effect. The next step of QuEChERS procedure is sample clean-up. This step uses dispersive solid phase extraction (dSPE) with the use of sorbents in various combinations. The function of the sorbents is to remove components from the sample matrix, such as fatty acids, carbohydrates, lipids, and various pigments (Micháliková et al., 2021; Pavkovich & Bell, 2019; Urban & Lesueur, 2017). Chromatographic methods capable of separating multicomponent mixtures, such as gas and liquid chromatography combined with mass spectrometry (GC-MS) (HPLC-MS), are required for the determination of pesticide residues. Chromatography equipment may differ in the type of stationary phase,

dosing inlet, column type, and detector. The combination of mass spectrometry with chromatographic techniques is required for the determination of pesticide residues because of its sensitive and highly specific nature compared to other chromatographic detectors (Pitt, 2009).

The aim of this review article is to provide an overview of analytical methods for monitoring pesticide residues in pollen samples. Some space is devoted to bee pollen composition to identify possible interferences around the whole analytical workflow. The main focus is laid on extraction techniques in the pollen sample preparation step and the discussion of their variability. The article deals with published results and methods developed for pesticide residues analysis from the point of view of separation method, mode of detection, and reached limits of detection. Key points of analytical methods needed to be considered in bee pollen analysis are critically evaluated.

Composition and bee pollen formation

Pollen is made up of a large number of pollen grains. The individual pollen grain is not visible to the naked eye, but its microscopic structure can be observed under a microscope. Pollen grain contains genetic information for the future sporophyte and is important in the reproduction and maintenance of the gene pool.

The transmission of pollen grain to the proximity of the flower's pistil is mainly ensured by wind or insects. As the stigma is sticky, the pollen grain is caught on it and sprouts (Dobrovoda, 1986). There are many other ways of transporting the pollen grain, for example, through birds, small mammals, or water. Flowering plants are divided into two groups, insect-pollinated plants and wind-pollinated plants. Both groups differ significantly morphologically.

The main differences between these two groups are that insect-pollinated plants have a vibrant coloration of flowers, and strong scent and produce nectar in large quantities to attract insects. The pollen created by these plants is heavy and sticky, making it easy to stick to the insect's body. Conversely, wind-pollinated plants do not show strong coloration (inconspicuous flowers) or smell and emit nectar only in small amounts. The pollen they produce is dry and dusty and in a much larger amount than the pollen of insect-pollinating plants. This pollen is very light, which ensures its spread by wind and its ultimate transfer to another plant (Chlebo, 2003; Dobrovoda, 1986).

The size of the pollen grain depends on the type of plant. The pollen grain has a size of approximately 2.5 μm to 250 μm as shown in Table 1. They are divided into six groups according to size from very small to gigantic (Grygorieva, 2018).

Other morphological features are the shape and colour of the pollen grain. There are many different shapes of pollen. Most often it is round. Other possible shapes include triangular, elliptical, oval, star, and so forth. The colour of pollen grains is characteristic of a given plant species. Within the same species, colour may be affected by the age of a flower or weather conditions such as temperature, humidity, and mineral nutrition. Plants can be of different shades of yellow, but rarely there are other colours such as blue, purple, pink, or red. Illustrative documentation of colour shades is bee pollen pellets. Various pigments such as carotenoids, flavonoids or anthocyanins are responsible for the colour of the pollen grain (Grygorieva, 2018). The colour shades of pollen pellets might be from various shades of yellow up to brown.

The chemical composition of pollen depends on many factors: the type of plant from which it originates, climate conditions and geographical area, and composition and moisture of the soil. Therefore, the values of chemical substances represented in individual types of pollen may differ according to reference (Ares et al., 2018).

Carbohydrates are the pollen components that are contained in it in the largest amount. Digestible carbohydrates make up 30.8% of pollen. Of this, up to 25.7% is reducing carbohydrates, especially glucose, fructose, and maltose (Komosinska-Vassev et al., 2015). In addition to reducing carbohydrates, there are also nonreducing carbohydrates such as sucrose (Rzepecka-Stojko et al., 2015).

Dry pollen contains approximately 22.7% protein, including 10.4% essential amino acids such as methionine, lysine, threonine, histidine, leucine, isoleucine, valine, phenylalanine, and tryptophan (Komosinska-Vassev et al., 2015). In addition to proteins, there is a number of nucleic acids, especially ribonucleic acids in pollen.

Of the lipids, which make up from 0.3% to 20% of pollen, it is important to mention unsaturated fatty acids such as palmitoleic, oleic, α-linolenic, and arachidonic acid. Among the saturated fatty acids identified in pollen are caproic, caprylic, lauric, myristic, palmitic, and stearic acids. The ratio of unsaturated to saturated fatty acids is 2.67. Another important part of pollen is essential unsaturated fatty acids which make up 2.7% of the content, which is reflected in its nutritional value. Also present are phospholipids 1.5% and phytosterols, about 1.1%. From the phospholipids, the most frequently present are the following: phosphatidylcholine, phosphatidylethanolin, phosphatidylositol, and the most popular phytosterols are the

Table 1. Size distribution of pollen grains.

Size	Range (μm)	Examples of species
Very small	<10	*Myosotis*
Small	10–25	*Sambucus nigra, Salis* spp.
Medium size	25–50	*Carpinus* spp., *Fagus* spp., *Quercus* spp., *Populus* spp.
Big	50–100	*Pinus sylvestris* L., *Pinus mugo Turra, Triticum aestivum* L.
Very big	100–200	*Picea abies* L., *Abies alba Philip Miller, Zea mays Linnaeus*
Gigantic	>200	*Cucurbita pepo* L.

following: β-sitosterol, campesterol (Komosinska-Vassev et al., 2015; Rzepecka-Stojko et al., 2015).

Phenolic compounds can be naturally found in nature, plants, and woody plants. Thus, they can also be found in pollen. Phenols, on average, make up 1.6% of pollen content. This group of compounds includes flavonoids, catechins, leukotrienes, and phenolic acids. Flavonoids are widely distributed in plants and they are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. They constitute 1.4% of pollen, most of which are kaempferol, quercetin, isorhamnetin. Chlorogenic acid is a potent antioxidant rich phytochemical widely found in plants and in pollen, it might be contained in the amount of 0.2% (Komosinska-Vassev et al., 2015; Rzepecka-Stojko et al., 2015).

In addition to nutrients, pollen also contains numerous biogenic elements: sodium, potassium, magnesium, calcium, phosphorus, manganese, iron, cobalt, nickel, copper, and zinc. Potassium and phosphorus are represented the most. Pollen is not only a valuable source of minerals but also a rich source of vitamins. Their content is approximately 0.7%. An important component is water-soluble vitamins, namely the group of B vitamins: B1 (thiamine), B2 (riboflavin), B6 (pyridoxine), and B7 (biotin). Acids, such as ascorbic, pantothenic, nicotinic, and folic acid, are an important part of bee pollen. Lipid-soluble vitamins make up only 0.1%. These are mainly vitamins A, E, D. Opposite to this, β-carotene is present in the largest amount, as well as other carotenoids (Komosinska-Vassev et al., 2015; Rzepecka-Stojko et al., 2015).

Field bees are involved in collecting pollen. They are 15- to 17-day old, though they can be younger. It is interesting that bees when flying through the air, create a positive static-electric charge on their body. This helps them to collect pollen because pollen has a negative charge, thus, it will be sticked to the body of the bee. In addition, when sitting on a flower, the field bee shakes the pollen off the anther with its sharp movements. The pollen then retains on the hair and the bee combs it into the pollen baskets (corbiculae) located on the last pair of its legs. The bee then moistens the pollen by secreting it from salivary glands or using nectar and forms it into so-called pollen pellets or pollen lumps. When the bee's corbiculae are full, it flies to the hive (Conrad, 2016; Dobrovoda, 1986).

After the bee arrives at the hive, it passes the pollen pellets either to the young bees or the pollen is already caught on the pollen catchers, and placed on the landing pad by the beekeeper. Some of the pellets are immediately consumed by the bees and some of them are also stored in brood comb cells. These cells are then partially filled with honey. When pollen pellets are stored in combination with honey and salivary secretions, lactic fermentation takes place. Thus, the bee ensures that the pollen remains preserved. In this form, it is easier to digest for bees. This is how the bee prepares it for its own consumption. Such pollen is called beehive/brood comb pollen, bee bread, or bee pollen perga (Dobrovoda, 1986; Včelí Peľ, 2016).

So far, the chemical composition of native pollen or pollen brought in pellets by the bees has been described. Brood comb pollen stored in a bee hive for several weeks has a different composition because it has been exposed to the bee's enzymes, honey and bacteria that have entered the cell (Conrad, 2016).

The summarization of knowledge on the pollen composition is an important base for the subsequent strategies for the analysis. For pesticide residues analysis, matrix interferences may be the principal complication for reliable pesticide analysis and markedly influences the detection and the quantification as well. Key interferences need to be removed in the sample preparation process; therefore, the whole analytical methods need to be proposed according to the matrix composition.

Analytical methods for detection and determination of pesticides in pollen

An overview of research papers devoted to innovative analytical methods discussing the detection and quantification of pesticide residues in pollen samples published between 2009 and 2021 is provided in Table 2. The summarised data include details on pre-treatment, extraction techniques to isolate pesticides from samples and subsequent purification of the extract, as well as data for the use of separation and detection techniques such as instrumental detail as well as the evaluation of methods performance in

Table 2. Overview of analytical methods for pesticide residues analysis in bee pollen including reported real findings.

Analyte	Sample	Pretreatment	Extraction	Cleaning procedure	Instrumentation and condition	LOQ *LOD	Real findings	References
428 pesticide residues	Pollen collected from 10 honey bee colonies of an experimental apiary located at the University of Córdoba	freezing	QuEChERS 2 g sample (0.8 g MgSO$_4$, 0.2 g NaCl, 0.2 g sodium citrate tribasic dihydrate, 0.1 g sodium hydrogen citrate sesquihydrate, 2 mL water, 4 mL ACN)	dSPE 2 mL extract (50 mg PSA, 50 mg C$_{18}$, 50 mg Zsep, 300 mg MgSO$_4$) Evaporation (50 µL ethyl acetate)	GC – MS/MS HP-5MS UI column (15 m × 0.25 mm, 0.25 µm) MP: (He, 99.999 %) Injector (splitless mode) Quadrupole system EI LC- MS/MS Zorbax Eclipse Plus C8, Agilent column (2.1 mm × 100 mm, 1.8 µm) MP A: (0.1 % formic acid, milliQ water) MP B: (ACN, 0.1 % formic acid, 5 % MilliQ water)	1.0 µg/kg	135 µg/kg (coumaphos)	Morales et al. (2020)
1 insecticide sulfoxaflor	Pollen from cotton collected in Bole, Xinjiang Uygur autonomous region (northwest China)	–	QuEChERS 2 g of sample (0.5 mL water,3 mL ACN, 0.5 NaCl)	Extract (0.1 PSA, 0.1 C$_{18}$) Extract evaporation to dryness in water bath under stream of N$_2$ (40 °C) Reconstitution (2 mL ACN) Filtration (0.22 µm nylon filter)	UPLC-MS Eclipse plus C18 column (50 mm × 2.1 mm, ID, 1.8 µm) MP A: (0.01 % formic acid, water) MP B: (ACN) Flow rate 0.2 mL/min, gradient elution	*17 µg/ kg (Application of sulfoxaflor during cotton flowering) * 14.2 µg/ kg (Application of sulfoxaflor before cotton flowering)	7.7 µg/kg –39.2 µg/kg	Jiang et al. (2020)
1 insecticide Spinetoram (J, L)	Bee pollen samples obtained from local markets (Valladolid, Spain)	Drying (45 °C) Grinding	QuEChERS 1 g sample (8 mL mixture of water: ACN (25:75, v/v), 1 g MgSO$_4$, 0.5 NaCl, 0.8 g trisodium citrate dihydrate) Cooling with dry ice (2 min)	dSPE 2 mL extract (150 mg MgSO$_4$, 10 mg PSA) Extract evaporation in rotary evaporator (40 °C) Reconstitution (1 mL mixture of ACN: ammonium formate (65:35, v/v)) Filtration (0.45 µm nylon filter)	LC-MS Kinetex® EVO fused-core type column C18 (50 mm × 2.1 mm, 2.6 µm, 100 Å) MP: (10 mM ammonium formate, water, ACN) gradient elution	7 µg / kg (spinetoram L) 10 µg / kg (spinetoram J)	–	Ruiz et al. (2020)

Table 2. Continued.

Analyte	Sample	Pretreatment	Extraction	Cleaning procedure	Instrumentation and condition	LOQ *LOD	Real findings	References
1 insecticide Flubendiamid	8 pollen samples from local markets (Valladolid, Spain)	Drying (45 °C) Grinding	QuEChERS 0.5 g sample (15 mL mixture of ACN: water (70:30, v/v), 1 g NaCl) Cooling with dry ice (4 min)	Extract (1 g EMR-lipid) Extract evaporation in rotary evaporator (60 °C) Reconstitution (2 mL methanol) Filtration (0.45 μm nylon filter)	LC-MS Gemini® C18, Phenomenex column (30 mm × 4.6 mm, 3 μm, 110 Å) MP: (1 mM acetic acid, water, methanol) Flow rate 0.5 mL/min, gradient elution	4 μg/kg	–	Bernal et al. (2019)
66 pesticides	189 pollen samples and 226 samples of beebread collected from five major beekeeping areas in China including the Southwest Area (SWA), the middle and lower reaches of the Yangtze River Area (MLAA), the middle and lower reaches of the Yellow River Area (MLEA), the Loess Plateau Area (LPA) and the Northeast Area (NEA)	–	QuEChERS 2 g sample (3 mL water, 10 mL 1 % acetic acid in ACN, 2 g of glass beads, 0.5 g MgSO$_4$, 2 g NaOAc)	dSPE 5 mL extract (PSA: C$_{18}$: MgSO$_4$: GCB 1:1:3:0.15) Filtration (0.22 μm nylon filter)	UPLC-MS/MS Ac18 Acquity EBH C18 column (2.1 mm × 100 mm, 1.7 μm) MP A: (mixture of MilliQ water: methanol (98: 2, v/v), 0.05 % formic acid) MP B: (methanol, 0.05 % formic acid) Flow rate 0.45 mL/min gradient elution GC-MS HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm) MP (He, 99.999 %) Injector (splitless mode) Quadrupole system EI	0.0008 – 0.53 ng/g *>1 ng/g (alachlor, beta cypermethrin, quizalofop-p-ethyl, fenvalerate, deltamethrin)	32 pesticides in samples 31 pesticide residues in samples of beebread	Tong et al. (2018)
63 pesticides	133 pollen samples collected from 45 apiaries located in different landscape contexts in Spain	–	QuEChERS 10 g sample (10 mL 1% acetic acid in ACN, 4 g anhydrous MgSO$_4$, 1 g ammonium acetate)	2 mL extract (50 mg PSA, 50 mg C$_{18}$) Filtration (PTFE 13 mm × 0.22 μm)	LC-MS Luna C18, Phenomenex column (15.0 cm × 0.21 cm, 3 μm) MP A: (10 mM ammonium formate, Milli-Q water) MP B: (10 mM ammonium formate, methanol) Flow rate 0.3 mL/min, gradient elution	* <2 ng/g <5 ng/g	14 pesticide residues 56.2 ng/g (coumaphos) 10.9 ng/g (fluvalinate) 17.6 ng/g (amitraz)	Calatayud-Vernich et al. (2018)

Table 2. Continued.

Analyte	Sample	Pretreatment	Extraction	Cleaning procedure	Instrumentation and condition	LOQ *LOD	Real findings	References
2 NN insecticides (Imidacloprid and thiamethoxam)	Pollen collected from crops of cotton grown from seeds coated with imidacloprid, thiamethoxam and pure water	–	QuEChERS 2 ± 0.01 g sample (10 mL ACN, 3 mL n-hexane, 4 g anhydrous MgSO$_4$, 1 g NaCl)	2 mL extract (0.0 5 g PSA, 0.15 g anhydrous MgSO$_4$) Evaporation to dryness in water bath under stream of N$_2$ (40 °C) Reconstitution (200 μL solution of ACN: water (25:75, v/v)) filtration (0.22 μm nylon filter)	UPLC-MS/MS BEH Shield RP C18 column (50 mm × 2.1 mm, 1.7 μm) MP: (0.05% formic acid, water, methanol) Flow rate 0.4 mL/min, gradient elution	* 0.05 ng/g (imidacloprid) * 0.14 ng/g (thiamethoxam)	1. 61 – 64.58 ng/g (imidacloprid) ND – 14.521 ng/g (thiamethoxam)	Jiang et al. (2018)
–	22 samples of bee pollen from hives with suspicious poisoning symptoms	–	QuEChERS 2 g sample (2 mL water, 5 mL ACN, 20 mL 10 mg/L internal standard TPP, 2 g anhydrous MgSO$_4$, 0.5 g NaCl, 0.5 g trisodium citrate, 0.25 g disodium hydrogen citrate sesquihydrate)	dSPE 3 mL extract (125 mg PSA, 125 mg Z-Sep, 750 mg MgSO$_4$) Acidification (10 mL 5% formic acid in ACN)	GC-MS HP-5MSUI capillary column (15 m × 0.25 mm, 0.25 μm) MP: (He, 99.999 %) Injector (splitless mode) ToF EI	1–1773 mg/kg	8 pesticides (chlorpyrifos, coumaphos, fluvalinate-tau, chlorphenvinfos, pyridaben and propylcresol, oxyfluorfen methoprene) 1–1207 mg/kg (oxyfluorfen) 212–1773 mg/kg (methoprene)	Hakme et al. (2017)
161 pesticides	53 bee pollen samples from 13 Polish regions	–	QuEChERS 5 g sample (10 mL mixture of ACN: water (1:1), 4 g anhydrous MgSO$_4$, 1 g NaCl, 1 g sodium citrate, 0.5 g disodium hydrogen citrate sesquihydrate)	dSPE 2 mL extract (300 mg MgSO$_4$, 60 mg PSA, 50 mg C$_{18}$) Filtration (0.45 μm nylon syringe filter) Acidification (30 μL solution of formic acid: ACN (5:95))	GC-MS/MS Rtx-5 MS 5%-phenyl-fused-silica capillary column, Restek, Bellefonte, PA, USA (30 m × 0.25 mm, 0.25 mm) MP: (He, 99.999 %) Injector (splitless mode) Quadrupole system EI	–	356 μg/kg (propiconazole)	Roszko et al. (2016)

(continued)

Table 2. Continued.

Analyte	Sample	Pretreatment	Extraction	Cleaning procedure	Instrumentation and condition	LOQ *LOD	Real findings	References
					LC-MS nonporous (Cortecs) C18 column, Waters (100 mm × 2.1 mm, 1.6 µm) MP A: (mixture of water: methanol (90:10, v/v), 4 mM ammonium acetate, 0.2 % acetic acid) MP B: (mixture of methanol: water (90:10, v/v), 4 mM ammonium acetate, 0.2 % acetic acid) Flow rate 300 µL/ min, gradient elution			
54 pesticide residues	48 crude pollen samples collected from eight provinces of China	—	QuEChERS 1 g sample (4 mL water, 2 g of glass beads, 10 mL mixture of 1% acetic acid in ACN, vortexing for 2 min at room temperature and for 10 min at −20 °C, 0.5 g MgSO$_4$ and 2 g NaOAc, 5 mL ACN)	dSPE 2.5 mL extract (evaporation at 30 °C, 500 µL methanol) Filtration (0.22 µm nylon filter)	UPLC-MS/MS Acquity BEH, Waters (ethylene bridged hybrid) C18 column (2.1 mm × 100 mm, 1.7 µm) MP A: (mixture of water: methanol (98:2), 0.05 % formic acid) MP B: (methanol, 0.05 % formic acid) Flow rate 0.45 mL/ min, gradient elution	* <0.5 ng / g * 0.5291 ng / g (aldicarb sulfoxide)	19 pesticides 4516 ng/g (carbendazim) 162.8 ng / g (fenpropathrin) 176.6 ng / g (chlorpyrifos) 316.2 ng / g (fluvalinate) 437.2 ng / g (chlorbenzuron) 79.00 ng / g (triadimefon)	Tong et al. (2016)
26 pesticides	145 pollen samples collected from ten beehives in the experimental apiary of Embrapa in Jaguariúna (Sao Paulo State, Brazil) 21 commercial bee pollen samples obtained from beekeepers in the region of Ribeirao Preto (Sao Paulo State, Brazil)	storing in a freezer at −16°C until analysis, drying in a circulating air oven (40–42 °C, 12 h), grinding, homogenization	QuEChERS 2 g sample (15 mL ACN, 4.0 g anhydrous MgSO$_4$, 1 g NaCl, 1 sodium citrate and 0.5 g Sodium hydrogen citrate sesquihydrate)	dSPE 1 mL extract (95 mg PSA, 750 mg anhydrous MgSO$_4$) Evaporation to dryness in a rotary evaporator (40 °C) Resuspendation (1 mL of ACN)	GC-MS/MS Rxi® 5 Sil, Restek capillary column (30 m × 0.25 mm, ID, 0.25 µm) MP: (He, 99.999 %) Injector (splitless mode) Ion trap (Saturn 2100T)	*100 ng/g 10–100 ng /g	18 /145 samples 25 ng/g (residues of pendimethalin) 4 samples 25 ng/g (bioallethrin) 7 /21 samples	de Oliveira et al. (2016)

(continued)

Table 2. Continued.

Analyte	Sample	Pretreatment	Extraction	Cleaning procedure	Instrumentation and condition	LOQ *LOD	Real findings	References
7 NN insecticides (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, thiamethoxam)	20 commercial bee pollen from local markets (Valladolid, Spain)	Drying (45 °C) Grinding	QuEChERS 1 g sample (2 mL water, 6 mL ACN, 1 g MgSO₄, 0.5 g NaCl, 0.8 g trisodium citrate dihydrate) Cooling with dry ice (2 min)	2 mL extract (150 mg MgSO₄, 25 mg PSA, 25 mg C₁₈) 1 mL supernatant Extract evaporation in rotary evaporator (60 °C) Reconstitution (1 mL mixture of methanol: water (80:20, v/v)) Filtration (0.45 µm nylon filter)	UHPLC-MS/MS Kinetex EVO C18 column (50 mm × 2.1 mm, 1.7 µm, 100 A °) MP A: (0.1 % formic acid, water) MP B: (0.1 % formic acid, ACN) Flow rate 0.3 mL / min, gradient elution	* 0.6 − 1.3 g / kg 2.1 − 4.0 g / kg	–	Valverde et al. (2016)
3 NN and residues (thiamethoxam, clothianidin, imidacloprid)	Bee pollen provided by the Regional Apiculture Centre of Marchamalo (Guadalajara, Spain)	Drying (45 °C) Grinding	QuEChERS 2 g sample (10 mL dichloromethane) Filtration (paper filter) Extract evaporation in rotary evaporator Reconstitution (1 mL mixture of water: ACN (1:1, v/v)) Filtration (syringe filter)	–	LC -MS C18 column (4 mm × 2.0 mm) MP A: (0.1 % formic acid, water) MP B: (0.1% formic acid, ACN) Flow rate 0.5 mL / min, gradient elution	*0.6 µg/ kg (thiamethoxam) *1.2 µg/ kg (clothianidin) *0.6 µg/kg (imidacloprid) 2 µg/kg (thiamethoxam 4 µg/kg clothianidin 2 µg/kg (imidacloprid)	1 sample (thiamethoxam) 9 samples (clothiadinin) 5 metabolites of thiamethoxam (TM-5, TM-6, TM-8, TM-13, TM-14) 2 metabolites of clothianidin (CM-8, CM-11) 4 metabolites of imidacloprid (IMI-4, IMI- 5, IMI-10, IMI-11)	Sánchez-Hernández et al. (2016)
253 pesticides	Pollen collected from 41 apiaries located in different Spanish regions	freezing	QuEChERS 2 g sample (4 mL ACN, 2 mL water, addition of citrate salts)	dSPE a) 4 mL extract (50 mg PSA, 50 mg C₁₈, 300 mg MgSO₄) b) freezing out + dSPE 4 mL of extract (50 mg PSA, 50 mg C₁₈, 300 mg MgSO₄) c) freezing out + dSPE 4 mL of extract (40 mg Z-Sep, 50 mg PSA, 50 mg C₁₈, 300 mg MgSO₄) d) freezing out + dSPE 4 mL of extract (50 mg PSA, 50 mg C₁₈, 300 mg MgSO₄ a SPE s Z-Sep (40 a 50 mg)	HPLC-MS/MS Agilent Zorbax EclipsePlus column with a reversed-phase C8 (2.1 mm × 100 mm, 1.8 µm) MP: A (ACN, 0.1 % formic acid, 5 % MilliQ water) MP: B (0.1% formic acid, 5% MilliQ water) 0.3 mL/ min, gradient elution GC-MS/MS Agilent Ultra Inert GC column, HP-5MS UI (15 m × 0.25 mm × 0.25 µm) MP: (He, 99.999 %) Injector (splitless mode) Triple quadrupole system	GC analysis 5–1000 µg/ kg LC analysis 4–400 µg/kg	5 and 50 µg/Kg	Vázquez et al. (2015)

(continued)

Table 2. Continued.

Analyte	Sample	Pretreatment	Extraction	Cleaning procedure	Instrumentation and condition	LOQ *LOD	Real findings	References
20 NN and fungicides + insecticides synergent piperonylbutoxid	Pollen samples from field bean, strawberry and raspberry plants collected during the period of blooming	Sifting (pore sizes 250– 45 µm)	QuEChERS 100 mg (± 5 mg) sample (400 µL water, 500 µL ACN, 250 mg mixture of MgSO₄ : NaOAc (4:1))	dSPE extract (50 mg Supel™ QuE PSA / C₁₈ / GCB, mixture of 150 µl ACN / toluene (3:1)) Spin filtration (0.22 µm) Evaporation to dryness under vacuum Reconstitution (mixture of 120 µl ACN / water (30:70, v/v))	UHPLC-MS / MS BEH C18 column, Waters (2.1 mm × 100 mm, 1.7 µm) BEH C18 VanGuard Precolumn, Waters (2.1 mm × 5 mm, 130 A° 1.7 µm) MP A: (95% water, 5% ACN, 5 mM ammonium formate, 0.1% formic acid) MP B: (95% ACN, 5% water, 5 mM ammonium formate, 0.1% formic acid) Flow rate 0.15 mL / min, gradient elution	*0.01–0.84 ng / g ≤ 1 ng / g (except of clothianidin, epoxiconazole) ≤ 3 ng / g (imidacloprid, trititconazole)	0.80 – 67 ng / g (4 residues of NN pesticides) 0.10–14 ng / g (9 fungicides)	David et al. (2015)
115 analytes of different chemical classes	Pollen collected from different areas of Greece	Cooling samples with ice-packs (0 °C) or dry ice (−78 °C) Homogenization	QuEChERS ACN, water, hexane, MgSO₄, NaOAc, PSA)	(PSA, MgSO₄)	LC–ESI-MS / MS ZORBAX Eclipse XDB-C18 column, Agilent (2.1 mm × 150 mm, 3.5 µm) MP: A (water, 5 mM ammonium formate, 0.1 % formic acid, 0.02 % ACN) MP B: (methanol, 5 mM ammonium formate, 0.1 % formic acid) Flow rate 0.3 mL/ min, gradient elution	*0.03–23.3 ng / g 0.1 to −78 ng / g	14 chemical compounds 6.1–1273 ng/g	Kasiotis et al. (2014)
60 pesticides including metabolites	pollen trapped from honey bees (Apis mellifera) from apiaries in five locations in Connecticut, USA	–	QuEChERS 5 g pollen sample, addition of water to volume 15 mL, addition of isotopically labelled standard, 15 mL of ACN, 6 g MgSO₄, 1.5 g sodium acetate, 150 µL acetic acid	10mL of supernatant, 1.5 mg MgSO₄,0.5 g PSA, 0.5 g C18, 2 mL toluene	LC-MS/MS Zorbax SB-C18, 2.1 × 150 mm, 5 µm column, MP: 12.5% methanol in water to 100% methanol. Both solvents have 0.1% formic acid IT MS, ESI	0.5–20 µ g/kg	imidacloprid, phosmet, chlorpyrifos, and carbaryl, Indoxacarb	Stoner and Eitzer (2013)

(continued)

Table 2. Continued.

Analyte	Sample	Pretreatment	Extraction	Cleaning procedure	Instrumentation and condition	LOQ *LOD	Real findings	References
8 NN insecticides (acetamiprid, clothianidin, dinotefuran, flonicamid, imidacloprid, nitenpyram, thiacloprid and thiamethoxam)	Blank pollens (13 samples) from honey bee hives at Worcester Massachusetts	Grinding into fine powder and mixed using mortar and pestle	QuEChERS 2 g sample (8 mL water, 10 mL ACN, 3 mL n-hexane)	dSPE 1 mL extract (50 mg PSA, 50 mg C₁₈, 150 mg MgSO₄) Reconstitution (200 μL solution of ACN: water (15: 85, v/v) Filtration through a 1.5-mL vials (0.2 μm nylon filter)	HPLC-MS/MS YMC ODS-AQ column (100 mm × 2.1 mm, 3 μm) YMC ODS-AQ precolumn (20 mm × 2.0 mm, 3 μm) MP A: (water, 5 mM ammonium formate, 0.1 % formic acid) Mobile phase B (mixture of ACN: water (95:5, v/v), 5 mM ammonium formate, 0.1 % formic acid) Flow rate 170 μL / min gradient elution	0.1 − 0.5 ng / g (dinotefuran, nitenpyram, clothianidin, flonicamid and tiacloprid) ≤0.5 ng / g (imidacloprid, acetamiprid and thiamethoxam) *0.03 ng / g (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid and thiamethoxam) 0.03 and 0.1 ng / g (nitenpyram and flonicamid)	–	Chen et al. (2013)
7 NN insecticides (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, thiamethoxam)	27 samples of corbicular pollen collected from apiaries located close to fruit orchards of two Spanish regions (Murcia and Castilla la Mancha)	Drying (45 °C), Grinding, storing in dark place (−20 °C)	QuEChERS 2 g sample (10 mL dichloromethane) Evaporation and reconstitution (1 mL mixture of water: ACN (1:1)) Filtration	–	LC-MS Eclipse plus C18 column (50 mm × 2.1 mm, ID, 1.8 μm)	* 0.4 − 2.8 μg / kg 1.2 − 9.1 μg / kg	2 samples 5.8 μg / kg (acetamiprid, imidacloprid)	Yáñez et al. (2014)
80 multiresidues pesticides and veterinary drugs, belonging to different chemical classes	130 samples of pollens	–	citrate QuEChERS 2 g of pollens 10 mL ACN + 8 mL H₂O	dSPE adding a small fraction of hexane in ACN	LC-MS/MS, a Nucleodur Sphinx RP-C18 (50 × 2 mm, 1.8 μm) column MP: (A) water with ammonium formate 0.3 mM and 0.05% formic acid and (B) methanol gradient elution ESI triple quadrupole analyser GC-MS DB-XLB capillary column.30m × 0.25 mm I.D., 0.25 μm film thickness splitless injection MP: He (purity 99.999%), flow of 1 mL/min EI, TOF	1.0 − 230.7 ng/g	2–2595 ng/g	Wiest et al. (2011)

Table 2. Continued.

Analyte	Sample	Pretreatment	Extraction	Cleaning procedure	Instrumentation and condition	LOQ *LOD	Real findings	References
171 pesticides and toxic metabolites	320 beebread samples, 28 trapped pollens from 13 apiaries owned by 11 different beekeepers and 2 pollen samples directly from flowers (California a Florida)		QuEChERS 3 g sample (100 mL solution of PCS, 27 mL extraction solution (44 % deionized water, 55 % ACN, 1 % glacial acetic acid), 6 g MgSO$_4$, 1.5 g anhydrous NaOAc)	1. For LC-MS/MS 1 mL extract (0.05 g PSA, 0.05 C$_{18}$, 0.15 g MgSO$_4$) 2. For GC-MS SPE 2 mL extract (250 mg GCB, 500 mg PSA, 0.80 g anhydrous MgSO$_4$, 3 × 4 mL solution of acetone: toluene (7:3, v/v))	LC-MS/MS Agilent Zorbax SB-C18 column (2.1 × 150 mm, 3.5 μm) GC-MS J&W DB-5MS capillary column (30 m × 0.25 mm ID, 2 μm)	*0.1 ppb–35 ppb (98 pesticides)	98 pesticides and residues (in 350 samples)	Mullin et al. (2010)
10 pesticides	Corbicular pollen collected from hives located in orchards of citrus trees	Homogenization in a mortar	QuEChERS 1 g sample (4 mL water, 5 mL 0.1 % acetic acid in ACN solution, two mixtures of salts: 4 g of a) acetate buffer, MgSO$_4$: NaOAc (4:1, w/w), b) citrate buffer, MgSO$_4$, : NaCl: sodium citrate dihydrate, disodium citrate sesquihydrate (8: 2: 2: 1, w/w/w/w)) UAE 1 g sample (0.5 mL water, 4 mL 0.1 % solution of formic acid in ACN) Aliquot of extract 1 mL (a) 150 mg MgSO$_4$, 50 mg PSA, 50 mg C$_{18}$ b) 150 mg MgSO$_4$, 50 mg PSA, 50 mg GCB) Filtration (0.22 μm nylon filter)	dSPE 1 mL extract (using one of the following sorbents: 200 mg alumina/ 200 mg PSA/ 200 mg C$_{18}$/ mixture of 100 mg PSA and 100 mg C$_{18}$) Filtration (0.22 μm nylon filter)	LC- MS/MS Kinetex F5, Phenomenex column (100 m × 3 mm, 2.6 μm, ID, 100 Å) 0.3 mL/ min MP A: (LC- grade water, 0.1 % formic acid) MP B: (0.1 % formic acid)	*<100 ng/g	–	García-Valcárcel et al. (2019)

Table 2. Continued.

Analyte	Sample	Pretreatment	Extraction	Cleaning procedure	Instrumentation and condition	LOQ *LOD	Real findings	References
Residues of nine OCP, ten OPP and seven PP pesticides	Pollen of pine, water lily, rose, cole flower, Papaver rhoeas, Schisandra chinensis Baill., camellia, and fresh camellia gathered in Anhui Province in China	–	UAE 5 g sample (50 mL petroleum ether) Filtration (paper filter) Filtrate concentration to nearly dryness using a rotary evaporator (40 °C) Residue dissolving (2 mL petroleum ether)	SPE Column (activated carbon + C18, 3 mL petroleum ether) Elution of adsorbed pesticides (10 mL solution of petroleum ether–ethyl acetate (95: 5, v/v))	GC- μECD HP-5 column (30 m × 0.25 mm, ID 0.25 μm) MP: (nitrogen, 99.999 %) Injector (splitless mode)	*>0.02 mg / kg	0.3–3.3 μg / kg (OCP) 1.0–19.1 μg / kg (PP) 1.1–197 μg / Kg (OPP	Zhang et al. (2012)
66 pesticides, including acaricides, fungicides, insecticides, nematicides, and some metabolites	554 pollen samples, 53 commercial apiary sites located in Italy	–	SLE 10 g of each pollen sample was extracted with acetonitrile/water	LLE + MSPD with hexane and combined with MSPD purification on PSA and Salts	LC-MS/MS	2.5–10 μg/kg	–	Tosi et al. (2018)
7 NN pesticides (dinotefuran, nitenpyram, thiamethoxam, clothianidin, imidacloprid, acetamiprid a thiacloprid)	Commercial bee pollen from different regions of Spain	Grinding, Storing in dark place (for 15 minutes)	SLE 5 g sample (10 mL pure water, 10 mL hexane, 10 mL ACN, 6 g MgSO₄, 3 g NaOAc,)	SPE 7 mL extract (around 1 mg MgSO₄) 6 mL extract (Supelclean ENVI-Carb II / PSA cartridge + 6 mL of ACN) Rinsing (3 mL ACN) Reconstitution (0.75 mL water) Filtration (0.20 μm nylon filter)	HPLC-MS/MS Hypersil GOLD column (C18) (100 mm × 4.6 mm, 5 μm) MP: (ACN) Flow rate 1.0 mL/ min, gradient elution	*0.2 − 2.2 μg/ kg 0.4–4.3 μg/ kg	1 sample from Valencia plus 2 residues 9 μg /kg (acetamiprid) 13 μg /kg (imidacloprid)	López-Fernández et al. (2015)

(continued)

Table 2. Continued.

Analyte	Sample	Pretreatment	Extraction	Cleaning procedure	Instrumentation and condition	LOQ *LOD	Real findings	References
8 OCP pesticides (LDN, HPT, HPO, ESA, DDN, EDN, ESB, MTC)	Bee pollen	Grinding	MSPD 0.5 g sample (0.5 g C$_{18}$, 500 µL ACN, homogenization, filtration (filter paper disc, 0.5 g PSA placed at the bottom of a polypropylene column (85 × 15 mm)) Elution (8 mL of ACN) Evaporation to dryness Reconstitution (1 mL isooctane) Freezing (< −10°C)	–	GC-MS Equity-5TM column (30 m × 0.25 mm ID, 0.25 µm) MP: (He, 99.999 %) Flow rate 1.0 mL/ min Injector (splitless mode, 1.5 min)	LDN *1.4 ng /g, 4.6 ng /g DDN *4.7 ng /g, 15.6 ng/ g EDN *14.7 ng /g, 49.1 ng/ g ESB * 15.5 ng/ g, 51.5 ng/ g HPT/HPO *<2 ng /g, <7 ng /g ESA * 23.2 ng /g, 77.3 ng/ g MTC * 2.3 ng /g, 7.6 ng/ g	–	Vazquez-Quintal et al. (2012)
OPP pesticides	Bee pollen	Grinding in mortar	MSPD 0.1 g sample + solid support (Florisil, TiO$_2$-PVI, TiO$_2$-PST or TiO$_2$) Pesticide elution (organic solvent) Evaporation to dryness Reconstitution (isooctane, 0.2 g Na$_2$SO$_4$) Freezing (−3 °C, 60 min)	–	GC-MS Equity-5 column (30 m × 0.25 mm ID, 0.25 µm) MP: (He, 99.999 %) Injector (splitless mode, 1.5 min) Quadrupole system	–	–	Torres-Perea et al. (2013)

(continued)

Table 2. Continued.

Analyte	Sample	Pretreatment	Extraction	Cleaning procedure	Instrumentation and condition	LOQ *LOD	Real findings	References
3 pesticides (diazinon, thiacloprid and difenoconazole)	Pollen and bee bread collected from combs inside the hives placed in two apple orchards established in three sites (Brdo pri Lukovici- Ljubljana, Čadovlje and Senično both in the Upper Carniola region)	–	MSPD 10 g sample (50 mL solution of acetone: petroleum ether: dichloromethane (1:2:2, v/v/v)) Evaporation to dryness under stream of N_2 (2 mL) Residue dissolving (8 mL mixture of cyclohexane: ethyl acetate (1:1, v/v))	gel permeation chromatograph Evaporation Addition (1 mL mixture of ethyl acetate: cyclohexane (GC-MS)/ 1 mL methanol (LC-MS/MS)	LC-MS/MS – GC-MS –	*0.01 mg / kg (thiacloprid, difenoconazole- LC-MS/MS) *0.02 mg / kg (diazinon- GC-MS)	1. and 16. day after application (Ljubljana) diazinon, thiacloprid difenoconazole 3. day after treatment (Ljubljana) ND (diazinon, thiacloprid) 6. day after application (Ljubljana) ND (thiacloprid) 6. day after application (Upper Carniola) Thiacloprid 10. day after application (Upper Carniola) ND (thiacloprid) 18. day after application (Upper Carniola) diazinon, thiacloprid	Škerl et al. (2009)
3 NN pesticides (thiamethoxam, acetamiprid, thiacloprid)	Bee pollen	–	Mini-SALLE 2 g sample (8 mL water, 10 mL ACN, 3 mL n-hexane, 10 µL internal standard, QuEChERS extraction salt	50 mg PSA, 50 mg C18 and 150 mg MgSO₄) Reconstitution (200 µL solution of ACN: water (15:85, % v/v) Filtration (0.2 um nylon filter)	HPLC Column C18 (4.6 × 150 mm, 5 µm) MP A: (water, 0.1 % formic acid) MP B: (methanol) Flow rate 0.8 mL / min, gradient elution	–	–	Tu and Chen (2020)

Notes: ACN: acetonitrile; C_{18}: octadecylsilyl; DDN: dieldrin; dSPE: dispersive solid phase extraction; ECD: electron capture detector; EDN: endrin; EI: electrospray ion source; ESA: endosulfan alpha; ESB: endosulfan beta; g: gram; GCB: graphitized carbon black; GC–MS: gas chromatography-mass spectrometry; HPLC: high-performance liquid chromatography; HPO: heptachlor epoxide; HPT: heptachlor; LC–MS: liquid chromatography– mass spectrometry; LDN: Lindane; $MgSO_4$: magnesium sulphate; min: minute; mini: SALLE– miniaturized salting–out assisted liquid–liquid extraction; mM: milli mol/L; MSPD: matrix solid–phase dispersion; MP: mobile phase; MTC: methoxychlor; NaCl: sodium chloride; NaOAc: sodium acetate; Na_2SO_4: sodium sulphate; ND: not detected; ng: nanogram; NN: neonicotinoid/s; OCP: organochlorine pesticide/s; OPP: organophosphorus pesticide/s; PCS: process control spiking; PP: pyrethroid pesticide/s; ppb: parts per billion; PSA: primary secondary amine; SLE: solid–liquid extraction; SPE: solid phase extraction; TiO_2: titanium dioxide; TiO_2–PST: titanium dioxide modified with polystyrene; TiO_2–PVI: titanium dioxide modified with polyvinylimidazole; TOF: time–of–flight analyser; TPP: triphenylphosphine; UAE: ultrasonic assisted extraction; v/v: volume per volume.

terms of limits of detection and limits of quantification. The reported detections and the concentration levels found in pesticide residues in real-life samples provide the need for analytical inspection. The next chapters will critically evaluate key points of the analytical methods and important contributions to the development of analytical methods for pesticides in bee pollen, principal investigations in the area of extraction, separation, and detection techniques are shown.

Sample collection, storage, and pretreatment

Bee pollen can be easily collected using bottom-mounted pollen traps. These are devices that can be placed in front of the entrance to the hive and contain openings large enough for the collected pollen to be squeezed through them. The principle of catching pollen is that the foraging bees entering the hive are forced to pass through a pollen catcher grid with holes that remove part of their pollen pellets held in the pollen baskets on the rear legs of the bees (Stoner & Eitzer, 2013).

Fresh pollen pellets contain 10–12% water and must therefore be dried thoroughly to approximately 4%. Dryers with an operating temperature of approximately 30 °C are used to preserve the pollen, but must not exceed 40 °C. If the pollen comes into contact with a temperature higher than 42 °C, thermolabile substances may be degraded. Sun drying can reduce pollen efficiency by up to 50% due to the oxidation of antioxidants. Contrary to this, some authors use a drying oven at 45 °C before the storage of samples (Valverde et al., 2016; Yáñez et al., 2014). Another way to treat pollen is to freeze or cool it immediately. After the pollen has dried, the next step is usually cleaning. This is because, in addition to pollen, various impurities, residues of wax, propolis, and small animals can also be present in the collected pellets (Conrad, 2016; Hoffmann, 2008; Hoover & Ovinge, 2018).

A special way of sampling was introduced by de Oliveira et al. (2016) comprising passive sampling with polyurethane foam discs.

Pollen samples are usually stored and frozen at around −20 °C after collection (Mullin et al., 2010; Vázquez et al., 2015) or kept in the dark at 4 °C until analysis (Bernal et al., 2019; Ruiz et al., 2020; Valverde et al., 2016). Alternatively, samples are preserved in the original packaging at laboratory temperature, spiked with pesticide standards and stored in the dark for stabilization (López-Fernández et al., 2015).

Sample extraction and clean-up

An important point of the analytical method development is the step of sample preparation in which it is necessary to isolate pesticides from samples. The composition of co-extracted components strongly depends on the sample matrix and markedly influences matrix effects at various stages of analysis; therefore, the clean-up of the extract needs to be optimized for each particular procedure. This is usually the most time- and labour-intensive step.

The sample treatment is mechanical processing and homogenization, isolation and concentration of analytes and ultimately the removal of interfering components. As a result, the sample becomes compatible with the separation and detection system (Micháliková et al., 2021). An overview of sample treatment techniques used for bee pollen analysis is depicted in Supporting Information Figure S1.

A basic method for the extraction is solid–liquid extraction (SLE), which combines the extraction of pollen by the organic solvent, e. g. hexane (López-Fernández et al., 2015). Due to the complexity of the pollen matrix, the QuEChERS procedure was most commonly used to extract pesticides from pollen samples. The QuEChERS method includes an extraction step with the addition of extraction solvent, usually acetonitrile (ACN), or acidified ACN and liquid–liquid distribution using extraction salts such as $MgSO_4$ and NaCl. The sample cleaning consists of dispersive solid phase extraction (dSPE) using different sorbents to minimize coextraction of materials and improve chromatographic efficiency.

For dispersive solid phase extraction, a variety of sorbents may be used. Primary secondary amine (PSA) is the most commonly used one and its main function is to remove components such as fatty and organic acids, carbohydrates, acidic components, lipids, and various pigments. PSA sorbent is not able to adsorb nonpolar pigments, therefore, graphitized carbon black (GCB) may be added to purify the extract. It is often added along with PSA and $MgSO_4$ as a part of the dSPE step. QuEChERS methods work well for most pesticides in nonfatty matrices such as vegetables and fruits. Samples with relatively high fat content may reduce the yield of more lipophilic pesticides (Pavkovich & Bell, 2019). Therefore, to isolate lipidic content, it is suitable to add silica gel modified by octadecylsilane (C_{18}) (Páleníková & Hrouzková, 2016). A multicombination of PSA sorbents with C_{18} and GCB is used to increase the efficiency of extract clean-up, especially in coloured samples (David et al., 2015; Tong et al., 2018). The addition of GCB to the mixture of C18 sorbent and PSA for dSPE significantly improved the detection limits for neonicotinoid (NN) analytes in different pollen species (David et al., 2015). Analyte recoveries were studied to determine whether the use of GCB and C18 would result in adsorption and loss of hydrophobic fungicides or fungicides with planar-

aromatic structures. The amount of 125 or 50 mg of a 1:1:1 mixture of sorbents was examined. The extract was centrifuged, filtered and dried in vacuum. Reconstitution was done with a mixture of 120 μL ACN and water. For some experiments using 50 mg of sorbents for the dSPE step, an additive extraction with a 3:1 mixture of 150 μL ACN and toluene was tested and it was shown that toluene can enhance the desorption of planar pesticides if GCB is used for clean-up (David et al., 2015).

In addition to these sorbents, a new group of zirconium-based sorbents has appeared on the market and has been successful in cleaning up various high-fat foods such as avocado, almonds and edible vegetable oils in pesticide residue analysis. Z-Sep consists of a mixture of C_{18} and silica coated with zirconium dioxide sorbents. The surface of zirconium dioxide contains Lewis's acid, Brønsted acid base, which, depending on the pH, may retain carboxylic acids and hard Lewis bases such as R-SO$_3$-, R-PO$_3$- a R-COO- (Hakme et al. 2017; Vázquez, 2015). Another newly discovered sorbent for better removal of complex matrices, namely Enhanced Matrix Removal lipid (EMR-lipid) was intended to remove phospholipids from various biological fluids from animal sources, including humans (Zhao et al., 2019). Before the dSPE step, freezing or cooling may also be used as a way of removing lipids from the sample (Schwartz, 2020).

Other types of additional sorbents such as alumina or EMR-lipid were tested by García-Valcárcel et al. (2019) and Bernal et al. (2019).

The official QuEChERS procedure according to AOAC 2007.01 was modified by Tong et al. (2016), and for analysis, the amount of 1 g of pollen samples was weighed and 4 mL of water was added to soak the material. PSA and GCB were used for the extract clean-up and the solvent exchange to methanol took place (Tong et al., 2016). Later on, authors Tong et al. in 2018 modified the weight ratio of the sample, particularly 2 g of pollen sample with 3 mL of water analysed. Combination of salts PSA: C18: MgSO$_4$: GCB (1:1:3:0,15; m/m) was applied by Mullin et al. (2010); Calatayud-Vernich et al. (2018) and a combination of methanol with n-hexane was used to re-dissolve the evaporated extract.

The standard CEN method based on citrate buffering appeared in several papers (de Oliveira et al., 2016; Hakme et al., 2017 Morales et al., 2020; Roszko et al., 2016; Valverde et al., 2016) and the modification for only 1 g of bee pollen sample was applied with the addition of 2 mL of water and 6 mL of ACN (Valverde et al., 2016). Individual differences in the quantity of samples and additions are shown in detail in Table 2.

A freezing method as a part of extract clean-up was reported by Vázquez et al. (2015). A portion of the supernatant was placed into dry-ice for 3 min, subsequently, the volume of 2 mL of extract was separated from the precipitate using a Pasteur pipette. Next, PSA, C18, and MgSO$_4$ were used for dSPE. Additionally, 1 mL of an aliquot of the above extract was percolated through an SPE cartridge containing 40 mg of Z-Sep (Vázquez et al., 2015). The combination of Z-Sep sorbent, PSA and C$_{18}$, along with freezing-out was reported by Morales et al. (2020) to monitor 428 pesticide residues. Freezing for lipid and protein precipitation was realized by Valverde et al. (2016) in a polystyrene box filled with dry ice for 2 min. Subsequently, the extract was separated from the precipitate and 2 mL was subjected to dSPE.

Adding a small fraction of hexane in ACN to eliminate lipids that interfere with mass spectrometry analysis was introduced by Mullin et al. (2010) and Wiest et al. (2011), followed by Chen et al. (2013) and Jiang et al. (2018).

Besides dSPE, the conventional SPE technique was routinely applied. The sorbent is usually a reversed stationary phase, such as C$_{18}$, or a polymer-based material (Páleníková & Hrouzková, 2016). Zhang et al. (2012) tested various combinations of SPE sorbents – C18, florist, activated carbon, activated carbon combined with C18, and activated carbon combined with florisil, resulted in the application of C18 sorbent combined with activated carbon showing the best performance and the lowest number of peaks of interferences.

Besides the QuEChERS procedure and its broad modifications, other extraction methods have been applied, such as matrix solid phase dispersion (MSPD), ultrasound assisted extraction (UAE), or miniaturized salting-out assisted liquid–liquid extraction.

The extraction technique of MSPD is widely applied to solid, semirigid or viscous samples. It extracts chemical and biological components from fruits and plants. It is also used to extract contaminants from food samples or the environment. MSPD is a technique that does not need a solvent extraction step. In MSPD, the sample may be mixed directly with the sorbent to obtain a homogeneous mixture. It is, then, transferred and placed in a SPE column, where it is washed and eluted with a liquid solvent (Pérez et al., 2020; Torres-Perea et al., 2013; Wen, 2020).

Vazquez-Quintal et al. (2012) chose a combination of nonpolar solid support, which was octadecylsilane (C18) and polar solvent ACN for extraction by MSPD. For extraction, 0.5 g of C$_{18}$ was inserted into the mortar and activated with 500 μL ACN. 0.5 g of pollen was added and homogenised. A filter paper disc

together with PSA has been placed on the bottom of the polypropylene column. The C18- blended pollen was packed over the PSA and compressed softly to eliminate air pockets. The matrix sample and pesticides were eluted with 8 mL of ACN from the column. The extract was collected in a vial and evaporated until dry under a gentle stream of nitrogen. Subsequently, the extract was reconstituted using 1 mL of isooctane and frozen below −10 °C for 12 h to precipitate high-molecular weight compounds. Finally, the extract was centrifuged and the supernatant was placed in vials ready for analysis. As the extraction conditions were simple, no internal standard was needed (Vazquez-Quintal et al., 2012). Torres-Perea et al. (2013) tested the following solid supports – Florisil, TiO₂-PVI (titanium dioxide modified with polyvinylimidazole), TiO₂-PST (titanium dioxide modified with polystyrene) or TiO₂ (titanium dioxide). Isooctane was used in reconstitution, in addition, 0.2 g of sodium sulphate was added to dry the extract.

Škerl et al. (2009) used 10 g of pollen sample for MSPD, centrifuged for 4 minutes together with 50 mL of acetone, petroleum ether and dichloromethane solution in a ratio of 1:2:2. The liquid phase was decanted, and evaporated at room temperature to approximately 2 mL and dried under a gentle stream of nitrogen. The residue was dissolved in a 1:1 mixture of cyclohexane and ethyl acetate.

A special technique derived from QuEChERS procedure and named a miniaturized salting-out assisted liquid–liquid extraction (mini-SALLE) was developed (Tu & Chen, 2020). In the extraction step, the pollen was crushed into a fine powder and 2 g of the sample was wetted by 8 mL of water. Subsequently, 10 mL of ACN, 3 mL of n-hexane, and 10 µL of the internal standard were added to the tube. The procedure applies salts for salting out, the top hexane layer was removed and 1 mL of ACN was transferred to a 2 mL dispersion SPE tube for clean up by PSA, C18, and MgSO₄. The supernatant was evaporated and the residue was reconstituted with a 200 µL mixture of ACN: water.

Ultrasound assisted extraction (UAE) uses high-frequency pulses (20 kHz) that pass through the solvent to form cavitation bubbles. The cracking of cavitation bubbles will cause a change in pressure and temperature, which will ensure an increase in the rate of transfer of the analyte to the solvent. The advantage of UAE is simplicity. It is less time consuming and requires less solvent than the other applied techniques (Jitan et al., 2018; Louie et al., 2020; Pacheco-Fernández & Pino, 2020). Zhang et al. (2012) used the amount of 5 g of pollen sample and extracted with ultrasound assistance with 50 mL petroleum ether. After filtration through filter paper,

25 mL of filtrate was concentrated to near dryness using a rotary evaporator. The residue was dissolved by the addition of 2 mL of fresh petroleum ether.

Separation and detection of pesticide residues

There are a few essential pre-requirements of successful and widely applicable pesticide residue analysis, namely high sensitivity leading to low detection and quantification limits and, for most applications, the multiresidual character of the method is an advantage. Rarely single-pesticide analysis could be done for some special purpose, for example, detection of flubendiamide in bee pollen (Bernal et al., 2019) or sulfoxaflor (Jiang et al., 2020). Owing to the complexity of pollen samples, robustness of the method is a key parameter which is tightly connected to the occurrence of undesired matrix effects. The choice of separation and detection technique is strongly influenced by the properties of the pesticides under scrutiny, predominantly their polarity and volatility. The most efficient approach to pesticide analysis involves the use of chromatographic methods due to the necessity to separate multicomponent samples. According to details summarized in Table 2, gas chromatography-mass spectrometry (GC-MS) (for details see Table 2) and the combination of liquid chromatography (LC) with tandem mass spectrometry (LC-MS/MS) is identified as the techniques most frequently applied.

GC-based techniques

In the majority of overviewed applications of GC separation of multicomponent mixtures, the 5% phenyl-polydimethylsiloxane capillary gas chromatography columns were utilized with various dimensions of columns and helium as the carrier gas. Spitless injection was exclusively used throughout the collected papers which corresponds with the need for ultra-trace level of pesticides detection.

Mass spectrometric (MS) detection is an important detection technique which satisfies present-day requirements for fast and reliable identification of compounds. The quadrupole analyser, the ion trap and the time-of-flight analyzer (TOF) were the most frequently used analyzers. The triple quadrupole analyzer and the ion trap may be used in MS/MS mode providing a high degree of selectivity and signal-to-noise ratio increase. Single ion monitoring (SIM) or multiple reaction monitoring (MRM) was used for the confirmation and quantitation of pesticides. In several cases of GC-based applications, detectors such as the microelectron capture detector (µECD) (Zhang et al., 2012) may be satisfactory and it may provide
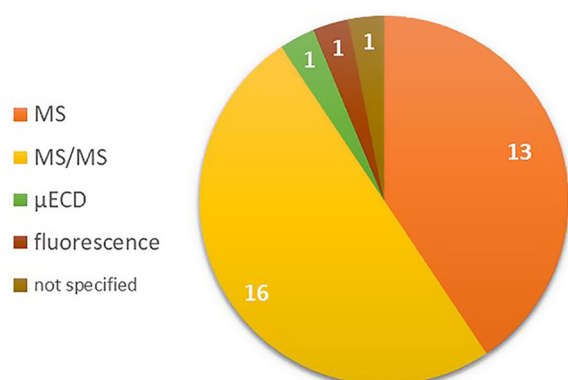
Figure 1. Distribution of detection techniques used for the detection of pesticides in pollen samples. Notes: MS: mass spectrometry; MS/MS: tandem mass spectrometry; µECD: microelectron capture detector.

lower detection limits of chlorinated compounds in comparison to MS detection. The distribution of detection techniques used for the detection of pesticides in pollen samples is shown in Figure 1. The developed analytical methods were evaluated in terms of linearity, limits of detection and quantification, accuracy, precision, extraction efficiency, the robustness of the method, and screening ability, by performing several tests. GC-MS systems can provide limits of detection as low as the low microgram level per kilogram of the sample. Therefore, LODs as a key validation parameters are summarized in Table 2.

GC-MS is currently the standard methodology for the determination of pesticide residues, due to its versatility, high selectivity, and unequivocal spectral evidence of the individual solutes. A significant aspect is a substantially lower price in comparison to LC-MS/MS.

LC-based techniques

The current trend of frequent application of more polar and less volatile degradable pesticides in the present-day agricultural practice has stimulated the development and application of liquid chromatography with mass spectrometric detection in pesticide residues analysis.

Meanwhile, separations were carried out mainly using C18-based analytical columns and mixtures of water and acetonitrile in different proportions as mobile phases. Alternatively, C8 stationary phases, and acetonitrile and formic acid or ammonium acetate with methanol mobile phases were also applied. In some cases, the pentafluorophenyl propyl column, Kintex F5, was used (García-Valcárcel et al., 2019).

Predominantly, electrospray ionization was applied and the majority of the utilized instruments used the tandem connection of quadrupole analyzers. The ion trap (de Oliveira et al., 2016; Stoner & Eitzer, 2013) and the time-of-flight MS (Hakme et al., 2017) were seldom selected.

For the detection of pesticides, the complementary use of the fluorescence detector could be beneficial (de Oliveira et al., 2016).

Matrix effects as a key complication in the pesticide detection and quantification

Every particular method has to mitigate some of the main sources of uncertainty such as the sample amount, volumes of extractant, recovery, and extraction conditions and these are evaluated during the method development. For the analysis of bee pollen, matrix effects were identified as the main source of problems leading to incorrect identification and/or quantification of pesticide residues. Matrix effects, expressed as the signal from the pesticides in the matrix compared with the signal in the solvent, are calculated as signal suppression/enhancement. Since the pollen matrix is very complex, significant ion suppression or enhancement of the analyte response may be observed. Therefore, to overcome this complication and to achieve precise quantification, matrix-matched calibration was used as recommended (Hakme et al., 2017 López-Fernández et al., 2015). Alternatively, matrix effects were evaluated by comparing the calibration curves obtained using fortified blank bee pollen extracts at various concentration levels and the calibration curves obtained at the same concentration levels using solvent (de Oliveira et al., 2016). For each concentration level, the matrix effect was expressed as the percentage of signal reduction or enhancement in the extract, relative to the standard prepared in a solvent. Due to the positive matrix effects observed, calibration curves were constructed using fortified blank bee pollen samples, compensating the matrix effects (de Oliveira et al., 2016). A standard addition method was done to take into account the possible matrix effect due to the different botanical origin of pollen collected by honey bees (García-Valcárcel et al., 2019). No matrix effects were reported for single-residue analyses (Bernal et al., 2019) with no need to make a correction or special approach to calibration. To identify matrix effects, matrix factors are calculated and evaluated during the method development and validation process for various apicultural samples such as pollen and pollen-based products (Blažková et al., 2022).

Real-life findings of pesticide residues

Developed analytical methods are used in real-life for the detection and control of various parts of the environment. Environmental monitoring was conducted using the analysis of 145 pollen samples collected from ten beehives in the experimental apiary in Sao Paulo State, Brazil and it was shown, that

bioallethrin and pendimethalin were identified in four and eighteen samples, respectively. They confirmed the need for environmental monitoring for the presence of pesticide residues. The potential to use bee pollen as a bioindicator of environmental contamination by pesticides was also confirmed.

High dose of sulfoxaflor found by Jiang et al. (2020) during flowering resulted in high residues in pollen and nectar. Risk assessments by contact exposure and dietary exposure showed that drip application of sulfoxaflor should have little negative effects on honey bees. Stoner and Eitzer (2013) proposed that concentrations should be reported as Hazard Quotients as well as in standard concentrations such as parts per billion. They used both contact and oral LD_{50} values to calculate Pollen Hazard Quotients (PHQ = concentration in ppb ÷ LD50 as µg/bee) when both were available. Pesticide PHQ ranged from over 75,000 to 0.01. The pesticides with the greatest PHQ at the maximum concentrations were (in descending order): phosmet, imidacloprid, indoxacarb, chlorpyrifos, fipronil, thiamethoxam, azinphos-methyl, and fenthion. PHQ as a means to evaluate the contamination was adopted by other researchers (Calatayud-Vernich et al., 2018). They proved the importance of analyzing pollen samples by reporting that one analyzed sample contained 10 different pesticide residues. Sixteen samples had more than 3 pesticides and an average of three pesticides per sample was detected. Interesting finding was observed by Calatayud-Vernich et al. (2018). Once pollen is stored in honeycombs, it can also be contaminated by other pesticides present in the wax. Tosi et al. (2018) discovered that pollen collected outside the hives by forager honey bees was only contaminated by pesticides applied in agriculture. Bee-collected pollen was shown to be a valuable tool for environmental monitoring and for the detection of illegal uses of pesticides.

Conclusions

Bee pollen has been a popular item in the human diet due to its nutritional and health properties mainly owing to the presence of several bioactive compounds such as amino acids, vitamins, proteins, and lipids. However, the unintentional content of some pesticide residues may cause undesired effects on living organisms and to various parts of the environment as well. Bee pollen was shown to be the optimal type of sample to serve as a bioindicator of environmental and agricultural contamination.

For this purpose, highly sensitive and selective analytical methods are broadly applied. The crucial part of every analytical method for pesticide residues detection in bee pollen is sample preparation, which aims to isolate the component of interest from the complicated sample; remove possible interferences from the bee pollen matrix and, very often, increase the concentration of the target pesticides to a concentration detectable by the instrumental detection systems, which follows the sample preparation step. The most popular extraction technique is currently the QuEChERS procedure with a variety of modifications in both extraction and clean-up step utilizing a variety of sorbents for selective removal of pollen matrix components interfering in pesticide residues quantification.

Gas chromatography and liquid chromatography are the two most applicable ways of pesticide residues separation which is followed by detection predominantly by mass spectrometry. Arrangements with tandem mass spectrometry are expanding and efficient instruments for multiresidue detection and quantification of pesticides are on the rise. Improvements and variability of MS analysers have made significant progress and at present, the triple quadrupole arrangement is a gold standard if reliable detection, as well as quantification, is required for low concentration levels of targeted pesticide residues. High resolution MS, for example, tome-of-flight (TOF), has become an interesting technique for untargeted screening of possible contaminants.

Real-life analyses demonstrate that bee pollen monitoring is a valuable tool for environmental monitoring of pesticide contamination, including the detection of illegal agricultural uses of pesticides. Thus, bee pollen was confirmed to be a suitable environmental bioindicator.

Disclosure statement

Funding

ORCID

Svetlana Hrouzková https://orcid.org/0000-0001-8628-4347

References

Ares, A. M., Valverde, S., Bernal, J. L., Nozal, M. J., & Bernal, J. (2018). Extraction and determination of bioactive compounds from bee pollen. *Journal of Pharmaceutical and Biomedical Analysis*, *147*, 110–124. https://doi.org/10.1016/j.jpba.2017.08.009

Bernal, J., Nozal, M., Martín, M., Bernal, J., & Ares, A. (2019). Trace analysis of flubendiamide in bee pollen using enhanced matrix removal-lipid sorbent clean-up and liquid chromatography-electrospray ionization mass

spectrometry. *Microchemical Journal*, *148*, 541–547. https://doi.org/10.1016/j.microc.2019.05.037

Blažková, I., Hrouzek, J., Szarka, A., Pócsová, T., & Hrouzková, S. (2022). Analytical methods for pesticide residues determination in propolis and propolis-based products. *Acta Chimica Slovaca*, *15*(1), 103–116. https://doi.org/10.2478/acs-2022-0012

Calatayud-Vernich, P., Calatayud, F., Simó, E., & Picó, Y. (2018). Pesticide residues in honey bees, pollen and beeswax: Assessing beehive exposure. *Environmental Pollution (Barking, Essex: 1987)*, *241*, 106–114. https://doi.org/10.1016/j.envpol.2018.05.062

Chen, M., Collins, E., Tao, L., & Lu, C. (2013). Simultaneous determination of residues in pollen and high-fructose corn syrup from eight neonicotinoid insecticides by liquid chromatography–tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, *405*(28), 9251–9264. https://doi.org/10.1007/s00216-013-7338-7

Chlebo, R. (2003). Opelovanie a včelia pastva. Poľnohospodársky poradenský systém. http://old.agro-poradenstvo.sk/zv/vcely/vcely_01.htm?start

Conrad, R. (2016). Bee pollen- an overview. In: Bee Culture-The Magazine of American Beekeeping. https://www.beeculture.com/bee-pollen-overview/

David, A., Botías, C., Abdul-Sada, A., Goulson, D., & Hill, E. (2015). Sensitive determination of mixtures of neonicotinoid and fungicide residues in pollen and single bumblebees using a scaled down QuEChERS method for exposure assessment. *Analytical and Bioanalytical Chemistry*, *407*(26), 8151–8162. https://doi.org/10.1007/s00216-015-8986-6

de Oliveira, R. C., do Nascimento Queiroz, S. C., Pinto da Luz, C. F., Porto, R. S., & Rath, S. (2016). Bee pollen as a bioindicator of environmental pesticide contamination. *Chemosphere*, *163*, 525–534. https://doi.org/10.1016/j.chemosphere.2016.08.022

Dobrovoda, I. (1986). 'Peľ', 'Včelie produkty a zdravie' (1ʳᵈ ed., pp. 99–126). Issued by Príroda.

García-Valcárcel, A. I., Martínez-Ferrer, M. T., Campos-Rivela, J. M., & Hernando Guil, M. D. (2019). Analysis of pesticide residues in honeybee (*Apis mellifera* L.) and in corbicular pollen. Exposure in citrus orchard with an integrated pest management system. *Talanta*, *204*, 153–162. https://doi.org/10.1016/j.talanta.2019.05.106

Grygorieva, O. (2018). Charakteristika pelu. In book: Peľ a včelie peľové obnôžky z niektorých druhov rastlín. https://www.researchgate.net/publication/330337626_Charakteristika_pelu

Hakme, E., Lozano, A., Gómez-Ramos, M., Hernando, M., & Fernández-Alba, A. (2017). Non-target evaluation of contaminants in honey bees and pollen samples by gas chromatography time-of-flight mass spectrometry. *Chemosphere*, *184*, 1310–1319. https://doi.org/10.1016/j.chemosphere.2017.06.089

Hoffmann, H. (2008). Anízovým olejom biologicky proti vijačke voštinovej. Včelárstvo, včely, med a ostatné veci okolo toho. http://www.pv.sk/vcely/mola_clanky001.asp

Hoover, S. E., & Ovinge, L. P. (2018). Pollen collection, honey production, and pollination services: Managing honey bees in an agricultural setting. *Journal of Economic Entomology*, *111*(4), 1509–1516. https://doi.org/10.1093/jee/toy125

Jiang, H., Chen, J., Zhao, C., Tian, Y., Zhang, Z., & Xu, H. (2020). Sulfoxaflor residues in pollen and nectar of cotton applied through drip irrigation and their potential exposure to *Apis mellifera* L. *Insects*, *11*(2), 114. https://doi.org/10.3390/insects11020114

Jiang, J., Ma, D., Zou, N., Yu, X., Zhang, Z., Liu, F., & Mu, W. (2018). Concentrations of imidacloprid and thiamethoxam in pollen, nectar and leaves from seed-dressed cotton crops and their potential risk to honeybees (*Apis mellifera* L.). *Chemosphere*, *201*, 159–167. https://doi.org/10.1016/j.chemosphere.2018.02.168

Jitan, S., Alkhoori, S., & Yousef, L. (2018). Phenolic acids from plants: Extraction and application to human health. *Studies in Natural Products Chemistry*, *58*, 289–417.

Kasiotis, K., Anagnostopoulos, C., Anastasiadou, P., & Machera, K. (2014). Pesticide residues in honeybees, honey and bee pollen by LC–MS/MS screening: Reported death incidents in honeybees. *The Science of the Total Environment*, *485–486*, 633–642. https://doi.org/10.1016/j.scitotenv.2014.03.042

Komosinska-Vassev, K., Olczyk, P., Kaźmierczak, J., Mencner, L., Olczyk, K. (2015). Bee pollen: Chemical composition and therapeutic application. https://www.hindawi.com/journals/ecam/2015/297425/

López-Fernández, O., Rial-Otero, R., & Simal-Gándara, J. (2015). High-throughput HPLC–MS/MS determination of the persistence of neonicotinoid insecticide residues of regulatory interest in dietary bee pollen. *Analytical and Bioanalytical Chemistry*, *407*(23), 7101–7110. https://doi.org/10.1007/s00216-015-8870-4

Louie, K., Kosina, S., Hu, Y., Otani, H., Raad, M., Kuftin, A., Mouncey, N., Bowen, B., & Northen, T. (2020). Mass spectrometry for natural product discovery. Comprehensive natural products III: Chemistry and biology (pp. 263–306). Elsevier.

Micháliková, M., Rusnáková, M., Hrouzek, J., & Hrouzková, S. (2021). Plynová chromatografia s hmotnostnou spektrometriou-metóda na analýzu včiel (pp. 64–65). Včelár.

Morales, M., Ramos, M., Vázquez, P., Galiano, F., Valverde, M., López, V., Flores, V., & Fernández-Alba, A. (2020). Distribution of chemical residues in the beehive compartments and their transfer to the honeybee brood. *The Science of the Total Environment*, *710*, 136288. https://doi.org/10.1016/j.scitotenv.2019.136288

Mullin, C., Frazier, M., Frazier, J., Ashcraf, S., Simonds, R., Vanengelsdorp, D., & Pettis, J. (2010). High levels of miticides and agrochemicals in North American apiaries: Implications for honey bee health. *PLoS One*, *5*(3), e9754. https://doi.org/10.1371/journal.pone.0009754

Pacheco-Fernández, I., & Pino, V. (2020). Extraction with ionic liquids-organic compounds. liquid-phase extraction. In Colin F. Poole (Ed.), Handbooks in separation science, liquid-phase extraction (pp. 499–537). Elsevier.

Páleníková, A., & Hrouzková, S. (2016). Súčasný stav a trendy extrakcie rezíduí pesticídov z nutraceutík. *Chemicke Listy*, *110*, 630–640.

Pavkovich, A. M., & Bell, D. S. (2019). Extraction/QuEChERS. In P. Worsfold, A. Townshend, C. Poole, & M. Miró (Eds.), *Encyclopedia of analytical science* (2nd ed., pp. 84–88). Elsevier.

Pérez, R. A., Albero, B., & Tadeo, J. L. (2020). Matrix solid phase dispersion. In C. F. Poole (Ed.), *Solid-phase extraction. Handbooks in separation science* (pp. 531–549). Elsevier.

Pitt, A. J. (2009). Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. *The Clinical Biochemist Reviews*, *30*, 19–34.

Roszko, M., Kamińska, M., Szymczyk, K., & Jędrzejczak, R. (2016). Levels of selected persistent organic pollutants