Review Article

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Latest trends in honey contaminant analysis, challenges, and opportunities for green chemistry development

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Abstract: The latest advances in honey trace contaminants analysis according to 70 articles gathered, mainly from the last 4 years, are critically reviewed, focusing on green and environmentally friendly aspects. Sample preparation protocols for multi-element analysis are slowly evolving towards green chemistry but older methods are still employed. Analytical methods are moving to mass spectrometry determinations, but other spectroscopic methods are also an answer. Dispersive sample preparation methods followed by chromatography coupled with tandem mass spectrometry proved their utility for multi-residue analysis of a wide array of trace organic compounds. Multiplex/multiclass methods development arises as a new field in honey contaminant analysis: They are greener than the traditional ones, as a bunch of families of chemical contaminants can be determined in a single extraction step. The regulatory framework did not follow the analytical procedures evolution. Honey is an animal-origin food, and contamination from other sources is seldom considered. The lack of holistic approaches from a legal point of view menaces public health as honey is consumed during the whole lifetime and hampers integrative analytical developments.

Keywords: honey, contaminants, trace analysis, green analytical methodologies

1 Introduction

Honeybees produce honey by gathering nectar from flowers or other sugar-containing natural secretions they find in the surroundings of the beehive. After cropping, honey is consumed and traded worldwide. The European Union Council Directive 2001/110/EC, and the *Codex Alimentarius* define honey as " the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from the secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature." Honey can be classified according to its origin (nectar or honeydew honey), mode of production and/or presentation (comb honey, chunk honey, drained honey, extracted honey, etc.), well as baker's honey (suitable for industrial uses or as an ingredient in other foodstuffs)" [1].

Honey is a complex mixture of compounds, some are common to all kinds of honey, such as sugars, organic acids, and vitamins, but minor compounds vary enormously according to the flora the bees visit. A recent high resolution mass spectrometry (HRMS) study detected more than two thousand possible compounds in Manuka honey. From these, 477 were common to chestnut, avocado, and eucalyptus honey, showing the intrinsic composition variability of honey regarding their origin [2].

Most of these natural components are at the $mg{\cdot}kg^{-1}$ level whereas the contaminants which are looked for are tenfold less concentrated. Because of that, acute toxic events due to contaminated honey consumption are unknown, but chronic exposure to them is of concern as honey is consumed by people of different ages and during their whole

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lives. This review focuses on the analysis of contaminants in honey from anthropogenic, microbiological origin, and natural toxins that threaten human health, stressing the environmentally friendly approaches for honey trace analysis. Analytically, two aspects must be considered: sample preparation and instrumental determination. In recent years, the number of new procedures for both steps has grown exponentially in complex matrices, and honey is not an exception. Honey sample treatment is a challenging issue. The advances in detection capabilities have boosted the development of new methodologies for determining honey contaminants, mainly based on mass spectroscopic measurements. New sensors and devices for the quick screening of pollutants are also of interest for in-field determinations but they are not widespread enough. In this review, only one work was found in this field as seen in Tables 1–5. Honey physicochemical parameters are evaluated to ascertain its rough quality. The usual honey quality parameters are moisture, pH, sugar (glucose, fructose, and sucrose), ash content, free acidity, hydroxymethylfurfural content, diastasic activity, and electrical conductivity [52,53]. A recent review highlights the major aspects of the analytical methods to evaluate the quality of honey production [54].

1.1 Honey contamination and its detection

Honey contamination has diverse origins, being either exogenous or derived from the apicultural practices used. Exogenous contamination comes with the food bees bring to the hive whereas veterinary drugs and acaricides are used inside the colony against parasites and harmful bacteria. External contamination arises from natural sources such as heavy metals, plant toxins, and anthropogenic contaminants like pesticides, persistent organic pollutants, and industrial products [55–57].

For the present work, 76 articles, reviews, and regulations were analyzed. The original scientific articles collected were 59, and most of them included the analysis of real, commercial honey samples ensuring the applicability of the methodology reported. The different contaminants are analyzed separately nowadays. Regarding the focus of the reviewed articles, 20 are related to the analysis of inorganic ions in honey, 16 to plant toxins, mostly Pyrrolizidine Alkaloids, five to the analysis of vet drugs, and just three to mycotoxins analysis and their occurrence. Pesticide residue analysis had the greatest development in the last decade and only seven references are mentioned, many of them devoted to the analysis of “difficult” pesticides, not amenable to the traditional multiple reaction monitoring (MRM) methods, but at least four comprehensive reviews were published covering the topic in the last 4 years. The occurrence of other organic contaminants (PAH and polychlorinated biphenyls [PCBs]) was studied in two different articles. Finally, seven articles were devoted to the actual trend of development of multiclass or multiplex methods in different matrices, in our case, honey. These methods cover different families of contaminants (residues of vet drugs, pesticides, and environmental contaminants as well as mycotoxins).

Besides the obvious differentiation between inorganic and organic contaminants, a unified vision of honey contamination is needed to seek food safety and consumer protection. Current advances in trace analysis allow this approach to support forthcoming new regulations. The conceptual framework of this article is shown in Figure 1.

2 Types of contamination: natural sources

2.1 Metals

Honey has 0.04–0.2% mineral content. Trace elements can be transferred from soil/water systems to flowers and therefore to honey; some of them are essential for life like copper, manganese, and zinc, which are involved in several physiological processes as cofactors of important enzymes. The different mineral profiles of honey have been used to discriminate them for their botanical and/or geographic origin [57]. Also, several heavy metals could be present and contaminate it. Particularly, rare earth and heavy metals originating from meteorites can be traced in honey [14]. The elemental fingerprint can be used to evaluate the adulteration of honey. The new trend for the accurate determination of metals in honey is to develop simple sample treatments, following green chemistry concepts [58,59]. Although both protocols are based on dispersive liquid–liquid microextraction (DLLME), thus minimizing the amount of solvent used for metal extraction, the strategy the authors followed was different. Sixto et al. used a mixture of organic solvents for Cd extraction, followed by flame atomic absorption spectroscopy determination. The method is fast and cheap, with excellent reproducibility at $10\,\mu g \cdot kg^{-1}$. It avoids the use of

Table 1: Methodologies for the analysis of heavy metals in honey applied or developed in the past 2–3 years

Contaminant	Sample preparation	Solvents	Time/*T*°C max	Detection technique	LOD*–LOQ** ($\mu g \cdot kg^{-1}$)	Sample's detection range, ($\mu g \cdot kg^{-1}$) (min–max)	Reference
Heavy metals	Microwave-assisted digestion	1 g Of sample HNO_3 cc and H_2O_2 30%	32 min/200	Inductively coupled plasma mass spectrometry (ICP-MS)	0.16–84**	As: ND–6.90 Cd: 0.38–6.47 Pb: 3.61–31.5	[3]
	Microwave-assisted digestion	0.5 g Of sample HNO_3 cc and H_2O_2 30%	45 min/180	ICP OES	1–10*	As: ND- 227.771 Cd: ND- 6.253 Cr: ND- 1433.23 Pb: 1.001–4.201	[4]
	Dry ashing	1 g Of sample NA	72 h/550	ICP OES	8–10**	Cd: <LOQ- 88 Pb: 14–1007	[5]
	Microwave-assisted digestion	1 g Of sample HNO_3 cc and H_2O_2 35%	Not reported	GFAAS	19–166**	Cd: <LOQ – 63 Pb: 120–651	[6]
	NA	0.1 g Of sample NA	320 s	Direct combustion analysis	0.05**	Hg: <LOQ - 1.71	[7]
	Wet digestion	0.2 g Of sample HNO_3 cc and H_2O_2 30% HCl cc and HNO_3 cc	30 min/95	ICP-MS Cold vapor atomic absorption spectrometry	1–10*	As: ≤ LOD Cd: 3–9 Pb: < LOD- 140 Hg: 0.7–3.2	[8]
	Dilution in HNO^3 1%	1 g Of sample in 10 mL of HNO_3 1%	NA	ICP-MS	0.28–2.46**	As: < LOD- 0.49 Cr: < LOD- 3.76	[9]
	Microwave-assisted digestion	0.5 g Of sample	30 min/190	ICP-MS	Not reported	As: 5.35–26.1 Cd: 1.15–10.80 Cr: 48.1–101 Pb: 16.5–42.8	[10]
	Microwave-assisted digestion	HNO_3 cc and H_2O_2 30% 0.5 g Of sample	2.5 h/210	ICP-MS	0.142–1.35**	As: 0.068–1.45 Cd: 0.056–9.17 Pb: < 0.325–18.1	[11]
	Microwave-assisted digestion	HNO_3 cc 1 g Of sample	60 min/ 120–160	GFAAS	25–168**	Cd: <LOQ-86 Cr: 660-2290 Pb: <LOQ-560	[12]
	Dry ashing	HNO_3 cc and H_2O_2 35% 5 g Of sample	12 h/600	ICP-MS	Not reported	Cd: ND-80	[13]

(Continued)

Table 1: *Continued*

Contaminant	Sample preparation	Solvents	Time/T°C max	Detection technique	LOD*–LOQ** (μg·kg^{-1})	Sample's detection range, (μg·kg^{-1}) (min–max)	Reference
		NA				Pb: ND-330	
	Microwave-assisted digestion	1.5 g Of sample	Not reported	ICP-MS	0.1–1**	As: 6.1–20	[14]
						Cd: <LOQ-11	
						Cr: <LOQ-19	
						Pb: <LOQ-14	
		HNO_3 cc and H_2O_2 30%					
	Wet digestion	0.5 g Of sample	Not reported	Microwave plasma atomic emission spectrometry (MP AES)	Not reported	Pb mean 2011–2018: 0.367 ± 0.0943	[15]
		HNO_3 cc and H_2O_2 30%					

* means that the value corresponds to the LOD and ** means that the value corresponds to the LOQ.

concentrated acids; no heating is necessary and it proved to be suitable for screening honey samples. Farisi et al. [58], on the other hand, employed a mixture of a ternary deep eutectic solvent (choline chloride, menthol, and p-aminophenol) and butanol followed by a salting out step to extract Zn, Cu, Co, Ni, Tl, and Pb from diluted honey. The organic phase was poured into distilled water and the organic phase was injected into the inductively coupled plasma-atomic emission spectrometry. The method has limits of detection (LODs) at 0.2–0.6 μg·kg^{-1} and limits of quantification (LOQs) at 0.7–2.4 μg·kg^{-1}. The method was tested in commercial samples. The solvents thus employed are less toxic than the conventional organic solvents. Despite this, conventional mineralization procedures are still the most widely used. However, improvements in the use of reagents can be made. A recent study optimized the amount of HNO_3 and H_2O_2 using a central composite experimental design to 3 and 1 mL, respectively, instead of the 7:30% relationship used traditionally. The use of mild microwave digestion employing less corrosive reagents is nowadays the preferred mineralization technique over traditional heating at 600°C [60]. Although the use of dilute acids in microwave-assisted digestion is a more environmentally friendly sample treatment, most of the reviewed works employ concentrated H_2O_2 and acids. The usefulness of the latter procedures is not in doubt, but avoiding the use of aggressive chemicals as the green chemistry concepts advice, needs further developments, with a focus on the protection of the environmental variables when a procedure is developed. Table 1 summarizes the metal determination methods employed in the last 4 years. For metal determination, one of the most used techniques is atomic spectrometry [57]. Depending on the levels in which metals are present, they can be determined by FAAS, MP, AES, ICP, OES, electrothermal atomic absorption spectrometry, and ICP-MS. Laser-induced breakdown spectroscopy emerged recently as a promising methodology since is rapid, has high efficiency, does not require sample preprocessing, and requires only a small volume of the analyte, being thus eco-friendly [61] but might not be appropriate for large-scale applications [62].

Some studies described the peculiar ability of honeybees to "filter" the nectar, reporting no relevant heavy metals levels in honey [63], but high levels of these toxic elements have been reported in honey from different regions [10,64]. *Codex Alimentarius* states that honey shall be free from heavy metals in amounts that can represent a hazard to human health, but there are no internationally established limits, only local regulations have been established. Maximum Residue Levels of 0.01 mg·kg^{-1} for mercury and 0.1 mg·kg^{-1} for lead were set recently in Europe [65,66].

Table 2: Methodologies for the analysis of pyrrolizidine alkaloids in honey applied or developed in the past 2–3 years

Contaminant	Sample preparation	Solvent/conditions	Detection technique	LOD*–LOQ** ($\mu g \cdot kg^{-1}$)	Sample´s detection range ($\mu g \cdot kg^{-1}$) (min–max)	Reference
Pyrrolizidine alkaloids (PAs)	Salting out assisted liquid–liquid extraction	25 g Of sample in 100 mL of water 10 mL was extracted with acetonitrile (ACN) $MgSO_4$ Na_2SO_4 pH 9.6	UHPLC-HRMS	0.07–0.22**	Scope: 118 alkaloids (59 PAs and 59 N oxides) Detections (only presence) in 64 out of 72 samples Concentrations not reported	[16]
	Solid phase extraction (SPE) (cation exchange)	10 g Of sample H_2SO_4 dil Eluent: MeOH/NH_4OH	UHPLC-MS/MS	1–3**	Scope: 18 alk–aloids (10 PAs and 8 N oxides) <100–2277	[17]
	SPE (mixed mode)	10 g Of sample in 30 mL H_2SO_4 0.05 M/MeOH (85:15) Eluent: MeOH/NH_4OH 15%	UHPLC-MS/MS	Not reported	Scope: 17 (12 PAs and 5 N-oxides) 0.2–281.1	[18]
	Direct alkaline dilution	0.5 g Of sample NH_4OH 6.5 mM Dilution factor 20	UHPLC-HRMS	10–20**	Scope: 30 alkaloids (18 PAs and 12 N oxides) Detections in 39 out of 80 samples LOQ-141.8	[19]
	SPE (cation exchange)	20 g Of sample in 20 mL 0.05 M H_2SO_4 Eluent: MeOH/NH_4OH 0.1%	HPLC-DAD	Not reported	Not found	[20]
	SPE (organosilyl-sulfonated halloysite nanotubes)	4 g in 40 mL 0.05 M formic acid Eluent: 100 mM formic acid in MeOH	UHPLC-MS/MS	1.9–3.6**	Scope: 4 PAs The method was not employed after its validation	[21]
	SPE (mixed mode)	1 g Of sample in 10 mL 0.15 M HCl Eluent: triethylamine, ammonia solution, methanol, and ethyl acetate, 0.1:0.1:2:8, v/v	Fluorescent lateral flow immunoassay	0.083*	Scope: 4 PAs Detections in 25 of 45 samples 3.24–46.47	[22]
	SPE (cation exchange)	2 g Of sample 0.05 M H_2SO_4 Eluent: MeOH/NH_4OH 3%	UHPLC-MS/MS	0.05**	Scope: 30 alkaloids (16 PAs and 14 N oxides) 365 detections in 490 samples LOQ- 182.25	[23]
	Quick easy, cheap, effective, rugged and safe (QueChERS)	2 g Of sample in 20 mL 50% acetonitrile containing 1% formic acid Anhydrous magnesium sulfate, sodium chloride, trisodium citrate, and disodium citrate	Nano LC- HRMS	0.05–2.5**	Scope:MS2 target ion screening approach 13 detections in 40 samples 0.14–74	[24]
	SPE (cation exchange)	5 g in 6 mL 0.05 M H_2SO_4	UHPLC-MS/MS	0.2**		[25]

(Continued)

Table 2: *Continued*

Contaminant	Sample preparation	Solvent/conditions	Detection technique	LOD*–LOQ** (μg·kg^{-1})	Sample´s detection range (μg·kg^{-1}) (min–max)	Reference
					Scope: 34 alkaloids (17 PAs and 17 N oxides)	
		Zn dust			349 detections in 775 samples	
		Eluent: MeOH/NH_4OH 5%			0.2–911.4	
	Salting out assisted liquid–liquid extraction	0.5 g Of sample	UHPLC-MS/MS	0.07–0.22**	Scope: 9 alkaloids (5 PAs and 4 N oxides)	[26]
		H_2O:ACN $MgSO_4{\cdot}7H_2O$ and Na_2SO_4 pH 9.6			69 detections in 71 samples LOQ to 37.3	
	SPE (cation exchange)	10 g Of sample in 30 mL 0.05 M H_2SO_4	Liquid chromatography-tandem massspectrometry (LC-MS/MS)	0.03–0.59**	Scope: 31 alkaloids	[27]
		Eluent: MeOH/NH_4OH 2.5%			More than 191 detections in 437 samples LOQ- 3313	

* means that the value corresponds to the LOD and ** means that the value corresponds to the LOQ.

The MERCOSUR in South America set maximum limits for As (0.3 mg·kg^{-1}), Cd (0.1 mg·kg^{-1}), and Pb (0.3 mg·kg^{-1}) content in honey [67], but Canada only suggests a limit of 0.1 mg·kg^{-1} to lead content [68]. All the instrumental methods described above are capable of reaching the legal requirements for the different heavy metals, as well as other inorganic contaminants. The knowledge of the levels of metals present in honey is of utmost relevance for environmental contamination evaluation and health risk assessment [14,64,69]. Besides the previous works [57], Squadrone et al. have recently demonstrated that the metal profile in honey is strongly influenced by geographical origin, the environmental conditions and it is also dependent on the floral type the bees visit [14]. Different strategies to evaluate the hazard to human health have been developed in the last years [10,12]. Scivicco et al. estimated carcinogenic and non-carcinogenic risks due to ingestion of honey in toddlers, adolescents, and adults based on the THQ, and, lifetime cancer risk finding potential carcinogenic risk for Ni, Cr, and As for all the groups. Mititelu et al. used a new methodology that calculated the corrected estimated daily intake (cEDI) taking into consideration the overall aggregate dietary exposure, the source hazard quotient for each metal (being the ratio between cEDI and acceptable daily intake), and the adversity-specific hazard index defined as the sum of the hazard quotients for the specific adversity. They found a moderate risk of nephrotoxicity, bone demineralization, cardiotoxicity, developmental toxicity, small decrease in body weight, or body weight gain after consumption of honey impurified with heavy metals. These works point to the necessity to continue with the risk assessment according to the levels of metals present and consumption habits in the region.

Regarding the comparison of metal content between conventional honey and certified organic honey, scarce information has been published. Bosancic et al. [70] found that lead content was less in certified organic honey. Lazarus et al. [11] reported a tendency of higher values in metal(oid)s content in conventional than organic chestnut, savory, and multi-floral honey but found a higher Cr content in organic honey. Again, it was pointed out that the environmental conditions should play a role in the honey´s elementary composition as well as the ability of the plants visited to take up heavy metals from the soil. These could be the reason for the different profiles found [14]. Leaching from the materials used in apiculture is another factor to be considered related to metal content together with botanical and geographical origin.

Table 3: Methodologies for the analysis of pesticides in honey applied or developed in the past 2–3 years

Contaminant	Sample preparation	Solvents/conditions	Detection technique	LOD*–LOQ** (μg·kg^{-1})	Sample´s detection range, (μg·kg^{-1}) (min–max)	Reference
Pesticides (38 pesticides and 5 related metabolites)	Magnetic solid phase extraction (MSPE) Magnetic polymer (N-vinyl pyrrolidone-divinyl benzene) (MVP-DB) as adsorbent	Dilution in 100 mL water	LC-MS/MS	0.002–0.1* and 0.5**	10 Samples analyzed	[28]
		20-min ultrasonication			Fipronil sulfone and imidacloprid were detected	
		Elution: 5 mL CAN–0.1% formic acid			0.65–1.37	
346 Pesticide residues	Modified QuEChERS acetate buffered	Acetonitrile extraction dSPE clean-up C18, primary and secondary amine (PSA) and MgSO4 sorbents	LC/MS-MS and GC/MS-MS	1–3* and 2–8**	100 Samples analyzed	[29]
					Coumaphos, thiamethoxam, N-(2,4-dimethylphenyl)formamide, piperonyl butoxide quantified in 42 samples	
					< MRL - 840	
9 Pesticides: OCs, OPs, neonicotinoids	QuEChERS	QuEChERS	Gas chromatography tandem mass spectrometry (GC-MS/MS) and LC-MS/MS.	Not reported	0.2–5.14	[13]
Glyphosate and aminomethylpho-sphonic acid (AMPA)	Water extraction	Water dilution	IC-HRMS Orbitrap	0.005–0.02**	32 Samples analyzed	[30]
		No pH adjustment			Glyphosate detected in 81% and 41% > EU MRL	
AMPA, glufosinate, glyphosate	Water extraction	Water dilution	IC-MS/MS	1.8–6.3**	Not reported	[31]
		no pH adjustment				
Pesticides	Dispersive solid-phase extraction with organic polymer (polystyrene)	eluent: hydrophilic deep eutectic solvents	LC-MS/MS	0.06–0.20* and 0.22–0.69**	Not reported	[32]
130 Pesticides and their metabolites	Modified QuEChERS method using C18, PSA, and Z-Sep	1 g sample	GC-MS/MS and LC-MS/MS	0.5–10**	63 Samples, 26% positive detections	[33]
					Predominant compounds detected: Coumaphos, imidacloprid, acetamiprid, amitraz metabolites (dimethylformamide [DMF] and *N*-(2,4-dimethylphenyl)-Nmethylformamidine [DMPF]), and tau-fluvalinate	
					1.3–785	
				10–200**	212 Samples	[34]

(Continued)

Table 3: *Continued*

Contaminant	Sample preparation	Solvents/conditions	Detection technique	LOD*–LOQ** (μg·kg^{-1})	Sample´s detection range, (μg·kg^{-1}) (min–max)	Reference
154 Pesticides and breakdown products	QuEChERS acetic/acetate-buffered multiresidue	Honey (20 g) diluted 1 in 2 with de-ionized water, 10 g of the solution was extracted	GC-MS/MS and LC-MS/MS		Low/negligible concentrations	
223 Pesticides	QuEchERS	Acetate-buffered acetonitrile extraction with Z-Sep + and PSA dSPE cleanup	LC-MS/MS and GC-MS/MS	1–10**	30 Samples, 90% positive findings Most frequent: thiacloprid, acetamiprid, carbendazim, DMF, total amitraz, thiamethoxam, thiacloprid-amide, dimethoate, azoxystrobin, tebuconazole, and boscalid 1–337	[35]
Small group OCs	QuEChERS modified solvent exchange to *n*-hexane	5 g honey, 1% acetic acid solution in acetonitrile and citrate salts, PSA dispersive solid phase extraction (d-SPE) cleanup	Gas chromatography with electron capturedetector	2.9** except heptachlor (5.6)	Not reported	[36]
121 Pesticides non-polar and polar pesticides	Water/acetone extraction, L–L cyclohexane/ethyl acetate, and GPC clean-up for non-polar-QuEChERS citrate-buffered extraction for polar pesticides	Multiresidue	GC-MS/MS and LC-MS/MS	Between 10 and 100**	61 Samples, all positive < MRL Coumaphos, amitraz and amitraz metabolite *N*-(2,4-dimethylphenyl) formamide detected	[11]
Persistent organic pollutants (POPs), pesticides Glyphosate, Glufosinate, and AMPA PCBs Polybrominated diphenyl ethers	POPs: pressurized liquid extraction methanol/water (1% formic acid).	POPs: 2 g honey Polar: 1 g honey	GC-MS/MS and IC-HRMS Orbitrap	10**	98 Samples Trace levels < MRL in all samples Glyphosate, Glufosinate, and AMPA not detected	[37]
6 OPs	restricted access material-molecular imprinted materials selective SPE	Better than conventional SPE	GC-FPD	0.5–1.9*	Not reported	[38]

(Continued)

Table 3: *Continued*

Contaminant	Sample preparation	Solvents/conditions	Detection technique	LOD*–LOQ** (μg·kg^{-1})	Sample´s detection range, (μg·kg^{-1}) (min–max)	Reference
400 Veterinary drugs and other contaminants	One-step extraction after separation by precipitation	5 g Of sample Precipitation buffer (pH, 5.0) Acetonitrile and ethanol (2:1, v/v)	LC-MS/MS	0.05–10*	60 Samples carbendazim (4 positive samples, 0.9–5.8 μg·kg^{-1}), indole-acetate acid (5 positive samples, 2.5–10.9 μg·kg^{-1}), N6-isopentenyl adenine (7 positive samples, 1.6–9.5 μg·kg^{-1}), acetamiprid (29 positive samples, 1.7–49.3 μg·kg^{-1}), chlorpyrifos (4 positive samples, 2.3–11.4 μg·kg^{-1}) and propargite (1 positive sample 5.9 μg·kg^{-1})	[39]
Chlorophenols	L-POF (Layered porous organic frameworks) adsorbent SPE extraction	10 g Honey diluted 100 mL, SPE procedure, cartridge preparation with L-POF	LC-DAD	0.5–1*	6 Samples 2.3–3.2	[40]
4 Acaricides	Method developed in 1996		Capillary LC-ultraviolet (LV)	22 and 1200 times lower than the narrow and normal-bore columns	Not reported	[41]
Antibiotics and 6 pesticides	A hybrid monolith of multi-walled carbon nanotubes (MWCNT)	MWCNT stationary phase	Nano-LC-UV-Orbitrap MS	ppb Levels	2 Samples, pesticides not detected	[41]

* means that the value corresponds to the LOD and ** means that the value corresponds to the LOQ.

Table 4: Methodologies for the analysis of antibiotics and other veterinary drugs in honey applied or developed in the past 2–3 years

Contaminant	Sample preparation	Solvent	Detection technique	LOD*–LOQ** (μg·kg^{-1})	Sample´s detection range (min–max)	Reference
Formic acid and oxalic acid	Sample dilution in water	5 g in 20 mL Deionized water with a resistivity of at least 18 MΩ Final dilution factor: 50	Ion chromatography coupled to a quadrupole Orbitrap mass analyser	200**	Formic acid: 469–779 mg·kg^{-1} Oxalic acid: 97–138 mg·kg^{-1}	[42]
Ciprofloxacin	Honey solution with deionized water	1 g of sample in 5 mL Deionized water with a resistivity of at least 18 MΩ	Electrochemical sensor	LOD = 4.96 μmol·L^{-1}	The method was not employed after its validation	[43]
64 Antibiotic substances	SPE (mixed mode)	2 g of sample	LC-MS-TOF	0.1–3.3* and 0.2–11**	Scope: amphenicols, lincosamides, macrolides, nitroimidazoles, pleuromutilins, quinolones, sulfonamides, and tetracyclines	[44]
Nitroimidazoles, quinolones and sulfonamides		HCl 2 M H_2O/Hexano			3 Detections in 55 samples Sulfonamides (sulfamethazine and sulfathiazole) and tetracyclines (oxytetracycline and tetracycline) at concentrations lower than 2 μg·kg^{-1}	
Amphenicols, lincosamides, macrolides, pleuromutilins, quinolones, tetracyclines and others	SPE (reversed phase)	Eluent: ACN/NH_3 30% 70:30, (v/v) 2 g of sample Na_2EDTA 0.1 M McIlvaine buffer pH 4 Eluent: MeOH				
Streptomycine	SPE	Water	enzyme-linked immunosorbent assay (ELISA)	7.7*; 17.8 ** For streptomycine and 5.5* and 13.5** for dihydrostreptomycine (calculated)	Scope: 24 antibiotics, six indicator PCBs	[11]
Dihydrostreptomycine	Liquid extraction	Eluent: serum diluting buffer 70:30 (v/v) Mixture of acetonitrile and acetone, evaporated and resuspended in Lab-Lemco broth	Premi®Test LC-MS/MS		61 Samples <LOQ	

(Continued)

Table 4: *Continued*

Contaminant	Sample preparation	Solvent	Detection technique	LOD*–LOQ** (μg·kg^{-1})	Sample´s detection range (min–max)	Reference
Sulfonamides	SPE	10 g Of sample in 20 mL 0.1M acetic acid at pH 5. 50 mL Of the acetone/dichloromethane mixture (1:1, v/v) NaCl, Na_2SO_4 Eluent: 5 mL of methanol that contained 2.5% ammonia				
Nitroimidazoles, Chloramphenicol, and Nitrofurans	d-SPE using MCX (mixed cation exchange) sorbent	Nitroimidazol: 5 g sample, Acetonitrile containing 1% acetic acid Sodium chloride, trisodium citrate dihydrate, and magnesium sulfate Nitrofuranes: 1 g sample 0.1 M HCl, derivatization with 10 mmol·L^{-1} 2-nitrobenzaldehyde 0.1 M Dipotassium hydrogen phosphate and 1 M sodium hydroxide 1:1 (v/v) Mixture of hexane and 1 mol Phosphate-buffered saline (phosphate buffered saline [PBS], pH 7.4)	LC-MS/MS ELISA	Not reported	48 Detections in 192 samples 0.2–5.528 μg·kg^{-1}	[13]
Tetracyclines	Liquid phase microextraction (LPME)	Vesicular supramolecular solvent (SUPRAS): didodecyldimethylammonium bromide (DDAB) and dodecyltrimethylammonium bromide (DTAB) under salt (NaCl) addition for coacervation	LC-UV	0.7–3.4*	1 of 2 Samples contained metacycline at 15.2 μg·kg^{-1}, as well as chlortetracycline at 12.2 μg·kg^{-1}	[45]
9 Nitrofuranes	ELISA	1 g Honey Hexane, 1 M HCl, and distilled water 2-Nitrobenzaldyde (10 mM) in dimethyl sulfoxide Dipotassium phosphate (0.1 M), NaOH (1 M), and ethyl acetate PBS 10 mM, pH 7.4	Nano-array. ELISA	0.19–0.9 μg·L^{-1}	The antibodies utilized do not allow the assays to reach the desired LODs Proof of concept	[46]

(Continued)

Table 4: *Continued*

Contaminant	Sample preparation	Solvent	Detection technique	LOD*–LOQ** (μg·kg^{-1})	Sample´s detection range (min–max)	Reference
Veterinary drugs, pesticides, and other contaminants	Dilution with water-non-targeted screening	0.2 g Sample acetonitrile:water (1:1) Dilution with water to 1% of honey (w/v)	HPLC-QTOF-MS,	Not reported (200 spike level for optimization)	40 Detections in 55 samples	[47]
400 Veterinary drugs and other contaminants	One-step extraction after separation by precipitation	5 g of Sample Precipitation buffer (pH, 5.0) Acetonitrile and ethanol (2:1, v/v)	LC-MS/MS	0.05–10*	60 Samples Mebendazole (1 positive samples, 1.2 μg·kg^{-1}), nalidixic acid (3 positive samples, 0.18–6.3 μg·kg^{-1}),	[39]
Veterinary drugs	87 Multiresidue Veterinary drugs	QuEChERS 5 g of Sample 0.2 g of ethylenediaminetetraacetic acid chelating agent solution Sodium sulfate, C18 and PSA	Nano-LC-HRMS	0.1–1*	The method was not applied in honey after its validation	[41]

* means that the value corresponds to the LOD and ** means that the value corresponds to the LOQ.

Table 5: Methodologies for the analysis of other contaminants in honey applied or developed in the past 2–3 years

Contaminant	Sample preparation	Solvent	Detection technique	LOD*–LOQ** ($\mu g \cdot kg^{-1}$)	Sample detection range, ($\mu g \cdot kg^{-1}$) (min–max)	Reference
Migrants from plastic packages	DLLME	1 g Of honey in 10 mL water	Gas chromatography mass spectrometry (GC-MS)	0.6–5.1*	Scope: 15 compounds	[48]
					8 Samples All the compounds were detected in all samples Oleamide: 115 and 275 dibutyl phthalate, bis(2-ethylhexyl) phthalate: 203 and 250 respectively bisphenol A 260	
		1.5 mL of AcN (disperser solvent) and 175 µL of chloroform (extractant solvent)		2–9.7**	Nonylphenol and *p-tert*-butylphenol 15.3 and 35.5	
PCBs	QuEChERS	10 g Of sample Modified QuEChERS Method 10 mL acetonitrile containing 1% Acetic acid Sodium acetate, anhydrous magnesium sulfate, and PSA	GC-microECD	5–10* and 10**	Scope: 11 PCbs 15 detections in 90 samples <LOQ-635	[49]
Polycyclic aromatic hydrocarbons (PAHs)	SPE C8	10% Methanol/water pH 10.	GC-MS/MS	0.5	Scope: 33 compounds	[34]
					212 samples Naphthalene was the most frequently found, in 18 of 212 samples (8.5%), followed by 2-methylnaphthalene (6 samples, 2.8%), phenanthrene (2 samples, 0.9%) and 1-methylnaphthalene (1 sample, 0.5%)	
		Eluent: ethyl acetate			<LOQ- the highest sum of the PAHs: 20	
	Ultrasound-assisted extraction	5 g Of sample + 8 g Extrelut *n*-Hexane/acetone Mixture (1:1) in an ultrasonic bath	LC-UV/Vis detector	Not reported	Scope: 16 PAHs 4 Detections in ten samples <LOQ: 12.58 sum	[50]
	DLLME and chemometric approach	2.5 g Of sample 5% Hydroalcoholic solution 150 µL Of chloroform 10 $g \cdot L^{-1}$ of NaCl	GC-MS	0.29–0.53* 1.05–2***	22 PAHs in 51 samples <LOD: 5.91	[51]

the symbol * means that the value corresponds to the LOD and the symbol ** means that the value corresponds to the LOQ.

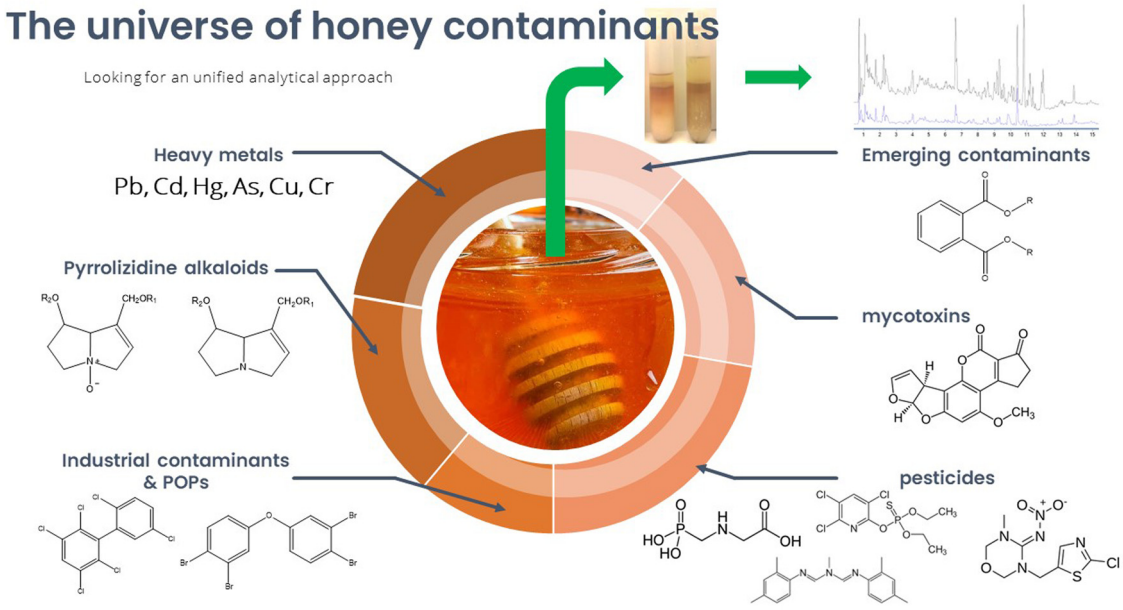

Figure 1: Different chemical contaminants that occur in honey. A unified analytical approach is needed to understand honey safety.

3 Plant toxins

3.1 Pyrrolizidine alkaloids (PAs)

A large group of secondary metabolites occurring in Asteraceae, Fabaceae, and Boraginaceae species are PAs. Their basic cores are the necines, platynecine, heliotridine, retronecine, and otonecine as shown in Figure 2. They are esters that occur in nature in two forms: the tertiary form and its corresponding N-oxides; PAs bearing a 1,2 unsaturation in the necine are hepatotoxic, carcinogenic, genotoxic, teratogenic, and pneumotoxic. The toxicity of unsaturated necines follows the sequence: macrocyclic, diesters, and monoesters. The increasing reports on PA contamination in foods, such as grain, milk, meat, eggs, or honey, stress the importance of performing a risk assessment on these alkaloids. The main drawback to gathering enough data for this study was the lack of appropriate analytical methods to determine them in food matrices as well as the absence of available standards. Despite that, interesting advances in PA determination have been performed in the last three years.

PA determination using GC-MS has been routine work in the past but the procedure is laborious and the real situation of the PA/PANO cannot be evaluated, as the N-oxides have to be reduced and the alkaloids derivatized. Within this framework, the global content of PAs in the sample is determined [71]. Most of the methods recently developed rely on solid phase extraction (SPE) sample treatments followed by LC-MS/MS PAs detection (Table 2). Simplified sample treatments were proposed based on the quick polar pesticide (QuPPe) approach [19,56]. Most QuEChERS ACN-based protocols failed to give good recoveries of pyrrolizidine alkaloids N oxides (PANOs) except the report by Rizzo et al. [26]. The PAs are analyzed generally in normal RPC-18 high performance liquid chromatography (HPLC)/ultra performance liquid chromatography (UPLC) columns, using formic acid/ammonium formate mobile phases. Under these conditions, the elution occurs in relatively short retention time (R_t) because alkaloids are positively charged, and the N-oxides are zwitterionic species. A workflow has been suggested using LC-MS (quadrupole ion trap) mass spectrometry (MS) instrumentation, in three analytical steps. The common product ion for all PAs is the ion at m/z = 120 Da and 138 Da for PANOs as shown in Figure 2. The parent ion experiment permits the detection of all the parent ions that yield these ions. In such cases, the operational mode of the tandem MS is Q1 (Full Scan Mode), Q2 (ramped), and Q3 selected ion monitoring mode. Then, an enhanced product ion experiment is performed. At the desired Rt, the ions are picked, and an MS^2 experiment is performed within the linear ion trap [56]. The MS^2 thus obtained is compared with the NIST library of Mass Spectra. In case there is no MS recorded, the structure can be elucidated to level 2 of the Schymanzki scale as in HRMS experiments. Then, the PAs and PANOs present in the sample are quantified through an MRM experiment and expressed as a known PA. The transitions are selected from the MS^2 spectra. However, it has been noticed that PANOs showed less sensitivity than PAs. All the works employ electrospray

ionization (ESI) in positive mode, as the alkaloid ions are already formed, but PANOs should protonate to be detected. The higher hydrophilicity of PANOs as well as their low basicity could be the reason for the lower sensibility detected of PANOs vs PAs in ESI + ionization conditions. HRMS methods were also developed (Table 2), allowing the simultaneous screening of target, suspect, and untargeted compounds. New targets of such a vast number of natural toxins can be detected and identified. It must be considered that the sole detection of an exact mass is not sufficient evidence for compound identification, as pointed out in the Shymanski rules. However, the new HRMS equipment, quadrupole-time of flights, and Q-Orbitraps^{R} allow a similar approach. These instruments employ different acquisition modes, which combined can yield relevant structural information about unknowns. Data Dependent Acquisition (DDA) and independent Data Acquisition (IDA) are acquisition modes that can be run simultaneously. Therefore, the fragmentation in the quadrupole can be followed whereas the exact mass of the parent compound is recorded at the same retention times.

The quantification of PAs and PANOs without a proper standard is a difficult task. Open chain diesters (acetyl lycopsamine) are tenfold more sensitive in ESI ionization than the more toxic cyclic diesters (retrorsine) and 2.6 times more than an open chain diester-N-oxide (echimidine N-oxide). For that reason, it was suggested that, in the absence of appropriate standards, the quantification has to be made considering the chemical family of PAs involved (monoesters, open chain diesters, cyclic diesters, and N-oxides of different esterification degrees). The point is relevant to risk assessment studies, as the relative toxicity of each group of 1, 2 unsaturated necines is different.

Despite concerns about their toxicity during chronic exposure, there is no *Codex* Standard for the maximum allowed levels in food, particularly in honey. However, the EU settled a default maximum residue level (MRL) of 0.05 mg·kg^{-1} of PAs in honey. Several studies have shown the presence of PAs in a high percentage (80–95) of retail honey [17]. Therefore, honey has the potential to be an important contributor to PA exposure. In recent years' risk assessment studies have been developed in New Zealand, China, Germany, and Romania [6,17,18,27]. The New Zealand study for drum honey concluded that the average lifetime health risk from PAs ingestion for the general population is not expected but there are many uncertainties, such as the differential toxicity evaluation of all PAs for the risk evaluation. A possible solution was presented in a Chinese study [18]. The potency of the different PAs was converted to lycopsamine toxicity units through their LD_{50}. It was found that 12% of the PA-contaminated Chinese retail honeys tested might pose potential health risks. In Germany, regionally produced unblended raw honey entails an increased exposure to consumers to PA/PANO, especially in children and high consumers [27].

More studies are needed to fill the existing gaps on the risks posed by PAs. Among them, are the relative toxicity potency between the different PAs and their N-oxides, the stability of PA/PANO in honey, the seasonal variation, storage time, etc. The lack of standards to properly quantify PAs and PANOs hampers their precise risk characterization. Also, the available methods based on LC-MS/MS have an elevated cost. Methods employing more simple

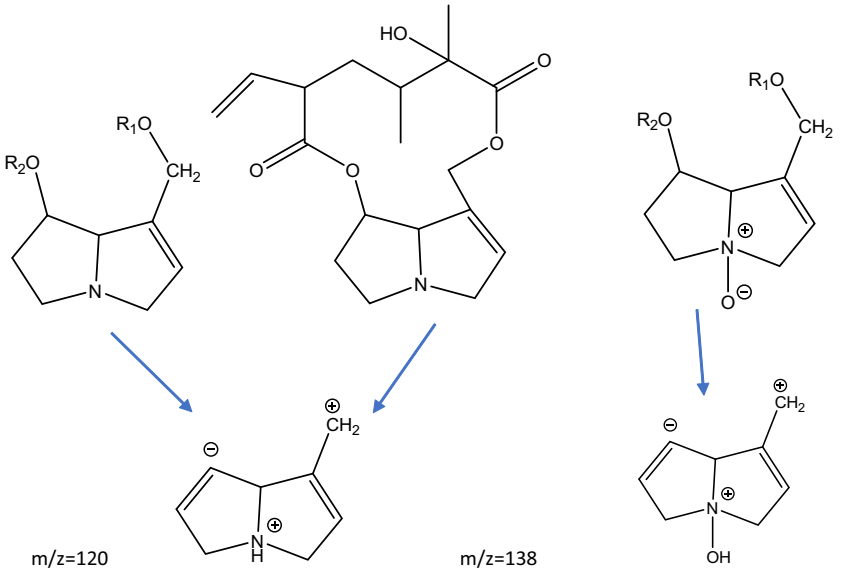

Figure 2: PA Cleavage into *m/z* 138 and 120 product ions.

equipment (HPLC with DAD detection) have been developed recently, and this can be considered an advantage in order to access to monitor these compounds at a low cost; however, it fails in the ability to detect the target compounds [20]. The advent of screening methods based on immunofluorescence which is in development could foster the spread of such studies [22].

3.2 Mycotoxins

Mycotoxins are low molecular weight (<1,000 Dalton) compounds produced mainly by molds of the genus *Aspergillus*, *Penicillium*, and *Fusarium*. They are frequently found in cereals and cereal-based food products and were detected in some types of honey from Turkey and Nigeria [72,73]. These analyses were performed by immunoassays, LC-UV, fluorescence detector, or tandem mass spectrometry following a traditional solvent extraction. Mycotoxins in honey have been analyzed following standard protocols, but the multiplex or multi-class analytical approaches have not been assayed yet in honey as a matrix, despite many examples of their application to other foods. The presence of Mycotoxins in honey is mostly related to bad post-harvest management by producers or economically motivated adulteration (EMA) fraud. When water is added, honey osmotic pressure is lower and molds are allowed to grow. Recent work in Turkish api products showed the presence of deoxynivalenol, T-2, and Ochratoxin A [73].

A Quechers-based protocol coupled to UPLC-MS/MS allowed the determination in 33% of honey samples of diverse botanical origin from Poland of seven mycotoxins as well as nicotine in the range 1–7 $mg{\cdot}kg^{-1}$ [74]. In this case, no relationship was found between the physicochemical parameters and mycotoxin findings. Honey from specific provinces of Nigeria showed up to 67 $mg{\cdot}kg^{-1}$ aflatoxin B1 [72].

Due to higher detection limits and ease of sample preparation procedures, the Elisa-based procedures for mycotoxin detection are the preferred ones, despite the fact of the intrinsic lack of proper molecular identification of the immunological method.

3.3 Anthropogenic sources: Pesticides

The presence of pesticides in honey and other bee products has been extensively studied in the European Union, the United States of America, and other countries during the past decade. These studies showed the ubiquitous presence of pesticides[55]. The main methodologies employed for multi-residue analysis which covers >100 analytes were LC-MS/MS and GC-MS/MS. Most of the sample preparation protocols applied were QuEChERS-based [75,76], and the trend continues, either for specific classes of contaminants or a higher number of them (>200) [35]. Most of the MRM methods for pesticide residue analysis for honey were developed during the past decade. All relevant chemical families of pesticides have been included in the scope of the method, achieving, in most cases LOQs below 0.01 $mg{\cdot}kg^{-1}$. QuEChERS methods are considered green methodologies [77]. The characteristics of the original method, which have been improved due to the sensitivity of the new instrumentation, such as the low amount of reagents consumption, reduced time analysis, and minimal waste produced, compensate well for the minimal use of a non-GRAS solvent such as ACN (10 mL or less). Substitution of ACN by other solvents like Ethyl acetate does not change the overall metrics to assess the QuEChERS greenness. Some pesticides are of particular concern for bee survival and the overall systems' sustainability. Neonicotinoids, fipronil, and its metabolites are particularly toxic for bees. The former at sublethal doses, hampering the orientation system of bees whereas the latter due to the very low LD50 as well as its persistence, has been responsible for massive bee death and honey contamination all over the world [55]. The analysis of 30 regional honey samples in Poland showed the presence of 15 different residues from 223 analytes investigated [13,36]. For the analysis of highly polar pesticides such as glyphosate, and glufosinate, QuPPe methodology has been widely adopted [78]. The presence of herbicides in honey has been overlooked, and scarce reports have taken notice of their presence in honey. Due to their low Kow, they are the most probable pesticide class to be found in honey. Fop family of herbicides, alachlor, and metolochlor, for example, are barely included in the scope of the MRM. Glyphosate presence in honey is nowadays a concern, due to the ubiquity of the diffuse contamination of the whole environment with this herbicide and the suggestion of having a negative impact on bee health through chronic exposure to it. This toxicity is enhanced when combined with some other currently found pesticides in honey. Glyphosate and AMPA can be analyzed through Ionic chromatography coupled to MS/MS Detectors [30,31]. Interestingly, honey dilution with water is enough to determine the herbicide and its metabolite, in a totally green process, but the cone of the MS/MS has to be cleaned very often as honey sugars caramelize on it. Thermo Fisher Scientific issued an application note to overcome this

problem by using a valve that drives to the waste the first section of the chromatogram, where the sugars elute. Other recent strategies involve molecular imprinting-based solid-phase extraction and nano- or capillary-LC. They are very interesting and promising but are limited to specific compounds or one family such as organophosphates or chlorophenols [40,41]. Accelerated Solvent Extraction has been used as an extraction procedure for the multiplex method for the analysis of PAHs; POPs and for pesticides such as glyphosate and glufosinate as well as amenable pesticides by multiresidue methods [41]. Table 3 shows the methodologies for the analysis of pesticides in honey applied or developed in the past 2–3 years.

3.4 Antibiotics and other veterinary drugs

Antibiotics and other veterinary drugs may occur in honey due to environmental contamination or beekeeping management practices. The analysis of multiclass antibiotics is performed by LC-MS/MS while specific compounds or groups are analyzed by immunoassays such as ELISA (Table 4). Radio inmuno assays have been used in the past and have been thoroughly reviewed. Recently, the inability of ELISA methods to detect the inactive isomers of chloramphenicol has been proven [79]. Nevertheless, due to their speed and reliable results, ELISA bench-top methods are used for the detection of sulfonamides, tetracyclines penicillin, and other antibiotics in honey. Besides this technique, recently molecularly imprinted polymers for the SPE extraction of specific antibiotics have been developed allowing the analysis of 7 macrolide antibiotics using hollow porous molecularly imprintedpolymers-based d-SPE and detecting two of them at 0.19, 0.53, and 1.7 $\mu g \cdot kg^{-1}$ [80]. Also, a low-cost paper-based electrochemical sensor for the detection of ciprofloxacin was developed with comparable figures of merit with other electrochemical techniques and high analytical frequency due to minimal sample preparation[43]. Multiresidue methods for antibiotics detection in honey have been able to analyze many antibiotics families such as amphenicols, lincosamides, macrolides, nitroimidazoles, pleuromutilins, quinolones, sulfonamides, and tetracyclines. Tested in real samples, three detections in 55 were reported [43]. A vesicular supramolecular solvent was used for the green liquid microextraction of tetracyclines. Among its green features are less consumption of extraction solvent, reduction of toxic reagents, minimization of energy consumption, small volume of analytical waste, and analysis of multianalyte system [44].

Synthetic acaricides used to protect the hive against varroa are usually detected through MRM methods but the most common and accepted acaricide in conventional and organic apiculture is oxalic acid. Ionic chromatography coupled with orbitrap mass spectrometry proved to have enough selectivity and specificity to detect oxalic acid in honey with very little sample preparation based on the QuPPe methodology [42]. Amitraz is a well-known acaricide frequently used in beehives and detected in beebrad, wax, and honey. The metabolites (DMF, DMPF, etc.) are quite polar and tend to migrate to honey, leading to detections in some cases in elevated levels above the MRL [11,33,35,55,81–86]. The residue definition of amitraz includes the sum of the original molecule and the metabolites derived from 2,4 dimethyl aniline. Older methods included a two-step SRM analysis of amitraz and its hydrolysis products, which led to an underestimation of the Amitraz content. LC-MS/MS determination after QuEChERS sample preparation proved to be a straightforward method for Amitraz and metabolites analysis. The metabolite concentrations are converted into molar equivalents of amitraz. Interestingly, the residue definition does not include the reaction's stoichiometry, and different results can be reported accordingly [55].

3.5 Miscellaneous (PCBs, PAHs, and Microplastics)

Polyaromatic hydrocarbons as environmental contaminants in honey have been detected in different studies performed in Italy, Australia, and the Herzegovina region [34,50,51]. On the other hand, persistent organic pollutants such as polychlorinated biphenyls have been found in studies performed in Brazil [49] and Italy [37]. As shown in Table 5, sample preparation was solvent extraction, QuEChERS, or DLLME methods followed by chromatography coupled by classic detectors mainly. Also, migrants from plastic food packages have been studied recently in honey. Targeted and untargeted analysis by GC–MS/MS was performed and proposed to monitor any contaminants of this type in honey. Fifteen target compounds, including styrene, phthalates, fatty acids, alkylphenols, and bisphenol A, were quantified. Untargeted analyses were also carried out, allowing other migrants in honey samples to be identified, such as two phthalates, four acids, three esters, one aldehyde, one hydrocarbon, and two alkyl phenol compounds [48].

3.6 Gaps and trends to assess honey integrity

The analytical methods for the determination of contaminants in honey are well-developed, of either inorganic or

organic origin. Nevertheless, there is room for the development of green chemistry-based protocols of sample preparation employing procedures such as ozonation or ultrasound and ionic liquids for the elemental analysis of metals and metalloids.

Honey elemental profile is useful to determine the origin of the samples but also serves to detect the presence of dangerous heavy metals. As plants take heavy metals from the soils, honey from heavy metal–contaminated areas should be strictly controlled.

The organic pollutants of honey have different origins. Bees are monitors of the environment and they gather all different types of organic molecules during their search for food. During the last few years, the presence of agricultural pesticides in honey has been extensively documented using variations of the QuEChERS methods, and they have been investigated in extensive pesticide monitoring campaigns [55]. Nevertheless, regulations all over the world are focused on the presence of veterinary drugs, mainly antibiotics and acaricides employed to fight Varroa mites. The European Union has settled the MRLs for many agricultural pesticides in honey, but *Codex Alimentarius* lacks this unified vision. The concept of E-MRLs that *Codex* [1] has developed for many commodities can be applied in the case of agricultural pesticides in honey. They are not associated with any particular good apicultural practice, but the evidence of their presence in honey and the potential risk to consumers' health is overwhelming and should be considered for honey safety.

A non-targeted LC-MS-based workflow for the identification of contaminants (mainly veterinary drugs and pesticides) belonging to different classes in honey has been optimized [47]. Recently, LC-HRMS has been applied for the sequential analysis of targeted analytes followed by suspect screening. This approach allowed the identification of various contaminants: pesticides, plasticizers, flame retardants, and additives. Also, markers of floral and geographical origin were identified in the same analysis [87]. This type of analysis is needed and has the potential to be expanded to many chemicals of interest following a risk-based approach or a "highly beneficial" compound search. However, this analytical technique is still highly costly and not easy to implement in routine labs.

4 Conclusions

ICP/MS protocols have been adopted increasing scope and sensitivity for inorganic origin contamination but the sample treatments are still based in non-greener procedures. More research is needed to adopt more environmental friendly procedures for sample treatment for inorganic species detection. Although HNO_3 digestion is firmly stablished and the amount and concentration of acid can be lowered, the avoidance of such aggressive reagents should be a must, despite sometimes the metrics for greener assessment giving acceptable results. As the analytical methodologies became more potent and the instrumentation increased in selectivity and specificity, the simultaneous detection of multiple families of contaminants (pesticides veterinary drugs, mycotoxins, POPs, industrial contaminants, and emerging contaminants) allowed the development of new multiclass/multiplex methods. Traditional boundaries that separate these contaminant families are crossed, showing the path to a more unified vision of the presence of contaminants in honey that will provide data for holistic risk assessment studies. These instrumental advance capabilities have not been accompanied in the regulatory field. As honey is considered an animal-origin product, the presence of agricultural pesticides is not always considered in the regulations. The same occurs with other anthropogenic contaminants and natural toxins. As laboratories seek the accreditation of the analytical procedures, only a change in the regulations can trigger the change in official labs. The regulation can foster the development of newer, green, multiplex methods to determine organic trace contaminants in a single analysis adopting a holistic vision. The establishment of safe MRLs for many of them in honey is far from being settled and harmonized.

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Conflict of interest: Authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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