Gas chromatography-tandem mass spectrometry multi-residual analysis of contaminants in Italian honey samples

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Gas chromatography-tandem mass spectrometry multi-residual analysis of contaminants in Italian honey samples

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ABSTRACT

Contaminants belonging to various classes, including polychlorobiphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs), pyrethroid insecticides (PYRs), fungicides (Fs), herbicides (Hs), synergists (SYNs) and insect growth regulators (IGRs) were analysed simultaneously in honey samples using a new simultaneous, easy and rapid method based on a liquid–liquid extraction with a mixture of *n*-hexane and ethyl acetate. It allowed recoveries in the range 80–137%, with limits of detection (LODs) between 0.10 and 5.21 ng g^{-1}, showing a good sensitivity and accuracy. All the analysed Italian honeys showed the presence of residues of OPPs; PAHs were in 46.8% of the samples and PCBs were always below the LODs; 53.2% of the samples were contaminated by OCPs, PYRs, SYNs and IGRs. In addition, 46.8% of the samples exceeded the maximum residue limits (MRLs) established by the European Community in honey for chlorfenvinphos (*cis* + *trans*), TPP, γ-HCH, tebuconazole, coumaphos and τ-fluvalinate (*cis* + *trans*).

KEYWORDS: Honey, organic pollutants determination, GC-MS/MS

Introduction

Honey is produced by bees from flower nectar or honeydew obtained from some plants. It consists of a solution of sugars, mainly fructose and glucose, with small amounts of higher sugars, enzymes, acids, salts, aromatic substances etc. It is possible to differentiate honeys through their botanical origin. Monofloral honeys are derived from a single plant species and have definite characteristics; while multi-flower honeys come from nectar of different plants. Finally, honeydew honeys are produced following the collection by bees of honeydew, a sugary secretion produced by certain insects (Viuda-Martos et al. 2008; Pohl 2009). Many honey uses are related to its therapeutic properties. The health benefits of honey are related to the digestive, respiratory and circulatory systems. It also shows antibacterial, antioxidant, antitumor, anti-mutagenic and anti-inflammatory properties (Al-Mamary et al. 2002; Alvarez-Suarez et al. 2010; Khalil et al. 2010). Unfortunately, several forms of contamination may affect this food. Some studies have reported the presence of organic residues in honey samples (Albero et al. 2004; Pirard et al. 2007). The prolonged use in agriculture of chemical compounds such as organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs), pyrethroid insecticides (PYRs), fungicides (Fs) and herbicides (Hs) can cause environmental contamination. The persistence of these substances on plants and soil can create shifts in the food chain. In effect, when bees collect pollen and nectar from plants, they may carry contaminants that can become incorporated into honey and other hive products. Moreover, the intensive use of chemical therapeutic treatments inside apiaries to control diseases can be considered

an extra source of direct contamination (Guillebeau 2004). In addition, polychlorobiphenyls (PCBs) are ubiquitous environmental contaminants as a result of uncontrolled spillage and atmospheric deposition linked to excessive use in the past (Gonzalez Sagrario et al. 2002). In addition, PAHs can be considered ubiquitous environmental pollutants, frequently observed in food (Dong & Lee 2009).

Commission Regulation (EU) No. 37/2010 establishes an MRL of 100 μg l^{-1} for coumaphos, Regulation (EC) No. 396/2005 and subsequent amendments (Regulations Nos 149/2008, 459/2010, 520/2011 and 899/2012) set an MRL of 0.05 mg l^{-1} for flusilazole, tebuconazole, cypermethrins, phosmet and ΣDDTs, and an MRL of 0.01 mg l^{-1} for diazinon, τ-fluvalinate, γ-HCH, endrin, endosulfan, chlordane and Σaldrin/dieldrin. The same regulation decrees a limit of 0.01 mg l^{-1} for all substances that are not specifically regulated.

The possibility that honey may contain various types of contaminants could decrease its quality. Many studies have been focused on the determination of various classes of contaminants in honey. Porrini et al. (2003) describe extraction of OCPs, OPPs, PYRs etc. from honey through organic solvents and a clean-up step; Kujawski et al. (2012) instead used a dispersive liquid–liquid microextraction; while Wang et al. (2010) used accelerate solvent extraction (ASE). The 'QuEChERS method' is the most universal extraction method used to analyse a wide range of compounds and generally involves the use of acetonitrile as a solvent for extraction. In particular, the efficiency of the QuEChERS approach for pesticide analysis in honey was validated by Blasco et al. (2011) and Calatayud-Vernich et al. (2016).

Liquid–liquid microextraction (Dobrinas et al. 2008) and ASE extraction with SPE clean-up (Lambert et al. 2012) have also been used to analyse PAHs in honey. Finally, Erdoğrul (2007) examined PCBs in honey samples by an extraction with hexane/acetone and Florisil columns. However, a simultaneous, easy and rapid analytical method for determining all these compounds in honey samples is not available in the literature.

The aim of this work was to analyse simultaneously 61 organic contaminants belonging to different classes, PCBs, PAHs, OCPs, OPPs, PYRs, Fs, Hs, SYNs and IGRs, by GC-MS/MS of honey samples from Sicily and Calabria (Italy). For this purpose, a convenient and easy multiresidue extractive protocol was developed.

Materials and methods

Chemicals

PCBs (a mixture of congener numbers 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189 at concentration of 2 μg ml^{-1}, and a mixture of congener numbers 28, 52, 101, 138, 153 and 180 at 10 μg ml^{-1}), PAHs (mixture EPA 525 PAH Mix A at the concentration of 500 μg ml^{-1}), triphenyl phosphate (TPP) and Supel™ QUE (with magnesium sulphate, sodium chloride, sodium citrate dihydrate and disodium citrate sesquihydrate) were purchased from Aldrich Chemical (Chicago, IL, USA); and Q-sep QuEChERS original extraction kits (with magnesium sulphate and sodium chloride) were bought from Restek Corporation (Bellefonte, PA, USA). To determine OCPs the Absolute Standard Inc. Mix were used consisting of α-HCH, β-HCH, γ-HCH, alachlor, aldrin, *cis*-chlordane, *trans*-chlordane, 2,4′-DDE, 4,4′-DDE, 2,4′-DDD, 4,4′-DDD, 2,4′-DDT, 4,4′-DDT, dieldrin, endrin and atrazine at a concentration of 100 μg ml^{-1}; ethyl acetate, *n*-hexane and acetonitrile for organic residue analysis were purchased from Fluka Analytical (Milan, Italy); ultrapure water was from VWR (Milan, Italy); bromophos-methyl, bupirimate, buprofezin, chlorfenvinphos, chlorpyriphos-ethyl, coumaphos, cypermethrin, diazinon, endosulfan II, flusilazole, τ-fluvalinate, phosmet, piperonyl butoxide, pyriproxyfen and tebuconazole were purchased from Dr. Ehrenstorfer (Augsburg,

Germany). In addition, commercial glucose syrup was bought from Karma (Salerno, Italy). Laboratory glassware was heated at 400°C for at least 4 h and was covered with aluminium foil prior to use. All the contaminants analysed are listed in Table 1.

Standard solutions

Stock standard solutions of individual standards were prepared by dissolving pure standard in *n*-hexane (at approximately 1000 mg l^{-1}); working standard solutions including all the examined compounds were prepared at 10, 25 and 50 ng ml^{-1} in *n*-hexane; calibration standard solutions were prepared at various concentrations in the range of 0.1–50 ng ml^{-1} in *n*-hexane. A standard solution of bromophos-methyl, used as an internal standard (IS), was prepared at 50 ng ml^{-1} in *n*-hexane. All the solutions were stored in closed vials at 4°C in a refrigerator.

Samples

Forty-seven samples of various types of honey were analysed in this work. The samples came from Sicily (the provinces of Messina, Catania, Agrigento, Caltanissetta and Trapani) and Calabria (the provinces of Reggio Calabria, Catanzaro, Crotone and Vibo Valentia), Italy, and were collected in 2012–13. The honeys were directly provided by beekeepers, who declared to have not performed any type of treatment directly in the hive in the last 6 months before collection. Each sample, contained in 500 ml glass jars with aluminium lids, appeared intact without signs of fermentation. The samples were 21 wildflower honeys, six chestnut honeys, four citrus honeys, three sulla honeys, three wildflower honeydews, three acacia honeys, two eucalyptus honeys, two orange blossom honeys, one thyme honey, one yarrow honey, and one chestnut and eucalyptus honey.

Sample preparation

Initially a modified form without clean-up of the method reported by Wiest et al. (2011) was tested. Thus, 5 g of honey were weighed, dissolved in 10 ml of ultrapure water and placed in a 50 ml centrifuge tube. To the dissolved sample, 10 ml of acetonitrile and Supel^{TM} QUE QuEChERS were added. The mixture was shaken manually for 1 min and subjected to a step in the centrifuge for 2 min at RT and 5000 rpm. Subsequently, 5 ml of the apolar phase were taken and then subjected to rotary evaporation at 30°C until a volume of 0.5 ml was obtained and, finally, internal standard bromophos-methyl was added.

Then the following method was tested: 10 g of honey were weighed, dissolved with 20 ml of ultrapure water and decanted into a 50 ml centrifuge tube; 10 ml of a mixture hexane/ethyl acetate 9:1 and Q-sep QuEChERS were added. After shaking manually for about 1 min, the sample was centrifuged for 5 min at RT and 5000 rpm. At the end, 5 ml of the organic phase were taken, reduced to 1 ml in a rotary evaporator at 30°C and, finally, reduced to a volume of 0.5 ml under a stream of nitrogen. Also in this case, no clean-up was necessary. Before instrumental analysis appropriate amounts of internal standard (bromophos-methyl 1 mg l^{-1}) were added.

Instrumentation and analytical conditions

Analyses were carried out using a Thermo Scientific Trace GC Ultra coupled with a triple quadrupole mass spectrometer TSQ Quantum XLS equipped with an autosampler TrisPlus RSH. Chromatographic separation was performed on a Supelco SLB-5 ms column, 30 m × 0.25 mm, with 0.25 μm of stationary phase. The carrier gas was He with a constant flow of 1 ml min^{-1}, the column oven temperature was 60°C for 1 min, 15°C min^{-1} until 120°C, and 10°C min^{-1} until 280°C (15 min final isotherm). The programmable temperature vaporising injector was set at 60°C (0.05 min),

14.5°C s^{-1} until 250°C (1 min), and finally a cleaning cycle at 320°C for 4 min. Transfer-line temperature was 300°C; injection volume was 1 µl; injection was in splitless mode for 1 min, after split 20 ml min^{-1}; source temperature (EI, 70 eV) was set at 250°C. Each compound was characterised by its retention time and two SRM transitions, the first used for quantification and the second as a confirmation. Variable collision energy experiments were performed on each transition to determine the optimum. All GC-MS/MS acquisition parameters are reported in Table 2.

Results and discussion

Analyses showed that all our honey samples were contaminated with at least one compound. Commercial glucose was used to prepare a matrix similar to honey in composition and physical properties for a blank, which was spiked with the standard mixture at the concentrations of 10, 25 and 50 ng ml^{-1}. The initial extraction procedure used acetonitrile and Supel™ QUE, as previous reported. Unfortunately, even though this protocol showed good recoveries for many compounds, only very low recoveries were achieved for PAHs (up to 11% for fluorene and up to 2% for acenaphtilene), probably due to the polarity differences between aromatic hydrocarbons and acetonitrile. Recent development in the QuEChERS methodology showed that a simplification of the traditional sample preparation is useful (Ohkawa et al. 2007), so Q-sep QuEChERS (with only magnesium sulphate and sodium chloride) was selected for extractive protocol. Furthermore, it has been demonstrated that the mixture of *n*-hexane and ethyl acetate (9:1) as an extraction solvent allowed good recovery for molecules of different polarity (Di Bella et al. 2006, 2010, 2014a; Lo Turco et al. 2007; Licata et al. 2012).

An extraction procedure using a mixture of *n*-hexane and ethyl acetate 9:1 showed higher recoveries for PAHs (up to 90% for benzo[k]fluoranthene and up to 125% for indeno[1,2,3-cd]pyrene). Therefore, all honey samples were analysed by this last protocol.

Quality assurance/quality control

High specificity was achieved using SRM analysis; all the examined contaminants could be detected without interferences, also in the case of co-eluting compounds.

In order to evaluate the procedure for the analytical protocol, the method was validating according to the European Union guidelines (SANCO/12571).

The method was tested to assess matrix effect, linearity, sensitivity, recovery, repeatability and precision.

Calibration curves (prepared in the range 0.1–50 ng ml^{-1}) were constructed with standards prepared in *n*-hexane and in blank extract. The obtained solutions were injected five times each.

The SRM1 peak area of all the contaminants was normalised against the peak area of bromophos-methyl (IS).

In order to evaluate the matrix effect, a comparison between a calibration curve derived from the standard solution prepared in neat solvent and the calibration curve derived from the standard solution prepared in blank extract was performed for each compound (Table 3).

A comparison between these two calibration curves, carried out according to Di Bella et al. (2014b), can be made by comparing the *t*-calculated value with the *t*-tabulated value for a confidence level of 95% ($\alpha = 0.05$) and the degrees of freedom corresponding to the two compared slopes. The *t*-tabulated value was 2.145. Then, as reported in Table 3, since for several analytes the *t*-calculated values were higher than 2.145, the matrix effect was not negligible. Because of this, the quantifications were carried out by using the calibration curves derived from the standard in blank extract.

Linearity was evaluated from the linear regression coefficients (R^2) of the calibration curves in blank extract. The obtained values were always above 0.9808 (Table 3).

The sensitivity of the method was estimated by establishing the LODs and LOQs. LODs were calculated from injections at the lowest detectable concentration (with a signal-to-noise ratio = 3) for each contaminant; LOQs were evaluated as peaks with a signal-to-noise ratio of 10. LODs were between 0.10 and 5.21 ng g^{-1}, instead LOQs were between 0.33 and 17.19 ng g^{-1} (Table 3).

Recoveries were calculated by using the blank sample spiked with the standard solutions at the concentrations of 10, 25 and 50 ng ml^{-1}. After 24 h the fortified blanks were subjected to the pretreatment procedures previously described. Recoveries, calculated by the average of five replicate analyses, were between 80% and 137%; the precision, expressed as RSD %, was satisfactory: always ≤ 20% (Table 3).

Intermediate precision was evaluated by analysing blank samples spiked with a standard solution at the concentration of 50 ng ml^{-1} after 10 days and after 40 days from the first analysis. Intermediate precision was in the range 1.1–6.2% at 10 days and in the range 3.8–8.7% at 40 days (Table 3).

Results from honey samples

The results obtained from the determination of organic contaminants in real samples are reported in Table 4. Substances found to be below their LODs are not reported. In the very few cases in which the residue quantification was out of the calibration range, a suitable further dilution of the real sample was made. OPPs were present in all samples; OCPs and other contaminants (Fs, PYRs, synergists (SYNs), insect growth regulators (IGRs)) were found in 53.2%; and PAHs were found in 46.8%. PCBs were absent from all samples.

According to the current legislation, 46.8% of the samples (22 of 47 samples analysed, 21 from Sicily and one from Calabria) exceed the MRLs. In particular:

- 100% of these samples exceeded the MRLs for chlorfenvinphos (*cis* + *trans*);
- 22.7% of the samples exceeded the MRLs for TPP; and
- 4.5% of the samples exceeded the MRLs for γ-HCH, tebuconazole, coumaphos and τ-fluvalinate (*cis* + *trans*).

It is evident that chlorfenvinphos (*cis* + *trans*) represents the major class of contamination in the analysed honeys. Despite the fact that various samples showed residues of TPP, γ-HCH, tebuconazole, coumaphos and τ-fluvalinate (*cis* + *trans*) above the MRLs, the same honeys all had chlorfenvinphos (*cis* + *trans*) levels in excess of the limit. We suggest an active role in contamination of honey from recycled waxes used for the construction of the frames, where the honey is deposited during its production. Beeswax could be a reservoir for the substances and particular conditions such as high temperatures in summer and/or prolonged periods of contact between honey and wax may favour dispersal of the contaminant and, consequently, the feedback in samples.

When comparing our data with other studies focused on individual classes of contaminants, the results are very different. In fact, Blasco et al. (2003) studied the presence of OPPs and OCPs in honeys from Spain and Portugal. Portuguese samples were more contaminated than Spanish ones and organochlorine compounds were particularly present with concentrations that ranged between 0.01 and 4.3 µg g^{-1}. Moreover, some acaricides and OPPs were identified with much lower concentrations than OCPs.

Albero et al. (2004) checked simultaneously 53 pesticides in Spanish honey; only three compounds were found with no chlorfenvinphos (*cis* + *trans*) in any sample. Lambert et al. (2012), when analysing 16 PAH residues in French honeys, highlighted a situation similar to that found our samples, characterised by levels between 0.030 and 5.800 mg kg^{-1}. Moreover, a similar comparison with a study from the Czech Republic shows analogous PAHs levels, even if qualitatively the variety of compounds that we identified is smaller (Batelková et al. 2012). Although the studies do not show excessive levels of PAHs in honey, they can be related to the surrounding environment, where human activities and the related pollution are the primary sources of contamination (Garban et al. 2002).

Conclusions

The application of our extractive protocol in combination with a sensitive GC-MS/MS technique made possible the accurate quantification of various classes of contaminants in honey, including PAH compounds. In particular, the use of appropriate extracting solvents such as hexane and ethyl acetate permits recovery and simultaneous analysis of many different substances at concentrations as far as 0.1 ng g^{-1}.

The mixture of *n*-hexane and ethyl acetate in the ratio 9:1 proved to be a good choice for the extraction of all compounds under analysis in this study because this is an optimal compromise between polarity and solvent strength. Even though the number of substances included in our study is not the highest, our analytical method is able to examine simultaneously the largest and the most diverse classes of contaminants. The use of a extracting mixture composed of *n*-hexane and ethyl acetate, in place of the more normal acetonitrile in the QuEChERS method, allowed us to achieve a multiresidue analysis capable of analysing compounds with widely different polarities. Moreover, the use of QuEChERS salts makes the protocol environmentally friendly, convenient and easy to use.

Moreover, the application of the developed method to a survey of various types of Italian honeys revealed the widespread presence of a large number of chemical contaminants in samples, especially in Sicilian ones. However, the major contamination was due to chlorfenvinphos (*cis* + *trans*), which was found in all the samples and exceeded the MRL in all 46.8% of honeys that contained contaminants in excess of at least one MRL. This kind of ubiquitous contamination might be related to the use of recycled beeswax in which chlorfenvinphos (*cis* + *trans*) could occur as a source of contamination (Wiest et al. 2011); the extensive and undisciplined use of this pesticide in the past may have caused its presence in wax and, consequently, migration into the produced honey, to create a significant quality and health problem.

Supplemental material

Gas chromatography-tandem mass spectrometry multi-residual analysis of contaminants in Italian honey samples

Disclosure statement

No potential conflict of interest was reported by the authors.

Additional information

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Table 1. Contaminants and their chemical class analysed in Italian honey samples.

Compounds	Class	Compounds	Class	Compounds	Class	Compounds	Class
PCB-28	PCB	PCB-189	PCB	Alachlor	OCP	*trans*-Chlorfenvinphos	OPP
PCB-52	PCB	Acenaphtilene	PAH	Aldrin	OCP	Triphenyl phosphate	OPP
PCB-101	PCB	Fluorene	PAH	*cis*-Chlordane	OCP	Phosmet	OPP
PCB-81	PCB	Phenanthrene	PAH	*trans*-Chlordane	OCP	Coumaphos	OPP
PCB-77	PCB	Anthracene	PAH	2,4´-DDE	OCP	Bupirimate	F
PCB-123	PCB	Pyrene	PAH	4,4´-DDE	OCP	Flusilazole	F
PCB-118	PCB	Benzo[a]anthracene	PAH	2,4´-DDD	OCP	Buprofezin	IGR
PCB-114	PCB	Chrysene	PAH	4,4´-DDD	OCP	Tebuconazole	F
PCB-153	PCB	Benzo[b]fluoranthene	PAH	2,4´-DDT	OCP	Piperonyl butoxide	SYN
PCB-105	PCB	Benzo[k]fluoranthene	PAH	4,4´-DDT	OCP	Pyriproxyfen	IGR
PCB-138	PCB	Benzo[a]pyrene	PAH	Dieldrin	OCP	Cypermethrin I	PYR
PCB-126	PCB	Indeno[1,2,3-cd]pyrene	PAH	Endrin	OCP	Cypermethrin II	PYR
PCB-167	PCB	Dibenzo[a,h]anthracene	PAH	Atrazine	H	Cypermethrin III	PYR
PCB-156	PCB	Benzo[g,h,i]perylene	PAH	Endosulfan II	OCP	Cypermethrin IV	PYR
PCB-157	PCB	α-HCH	OCP	Diazinon	OPP	*cis*-τ-Fluvalinate	PYR
PCB-180	PCB	β-HCH	OCP	Chlorpyriphos-ethyl	OPP	*trans*-τ-Fluvalinate	PYR
PCB-169	PCB	γ-HCH	OCP	*cis*-Chlorfenvinphos	OPP		

Note: PCB, polychlorinated biphenyl; PAH, polycyclic aromatic hydrocarbon; OCP, organochlorine pesticide; OPP, organophosphorus pesticide; H, herbicide; IGR, insect growth regulator; SYN, synergist; PYR, pyrethroid insecticide; F, fungicide.

Table 2. GC-MS/MS acquisition parameters.

Compounds	RT	SRM 1 (Q)	CE	SRM 2 (C)	CE
Acenaphtilene	10.16	152–150	40	152–126	40
Fluorene	11.71	166–164	30	166–139	30
α-HCH	12.89	181–145	9	219–183	9
Atrazine	13.33	215–200	15	200–104	18
β-HCH	13.42	181–145	15	219–183	10
γ-HCH	13.60	181–145	15	219–183	10
Diazinon	13.71	304–179	18	179–137	20
Phenanthrene	13.93	178–176	30	178–150	35
Anthracene	14.05	178–176	30	178–150	35
PCB-28	14.78	256–186	17	258–186	17
Alachlor	14.87	188–160	14	161–146	14
PCB-52	15.44	290–220	17	292–220	17
Chlorpyriphos-ethyl	15.65	197–169	8	199–171	8
Aldrin	15.80	263–193	20	293–258	17
Bromophos-methyl (IS)	16.11	331–316	9	329–314	9
cis-Chlorfenvinphos	16.25	323–267	13	267–159	13
trans-Chlorfenvinphos	16.48	323–267	13	267–159	13
cis-Chlordane	17.01	373–266	20	410–375	20
2,4´-DDE	17.02	246–176	20	318–246	20
PCB-101	17.11	324–254	20	326–256	20
trans-Chlordane	17.26	373–266	21	410–375	18
Pyrene	17.28	202–200	35	202–174	35
4,4´-DDE	17.63	246–176	30	318–246	30
PCB-81	17.65	290–220	18	292–220	18
Bupirimate	17.70	316–208	18	273–193	18
Flusilazole	17.71	233–165	18	233–151	18
Buprofezin	17.74	172–57	18	305–172	14
2,4´-DDD	17.77	235–165	25	237–165	18
Dieldrin	17.79	263–193	24	263–228	18
PCB-77	17.85	290–220	18	292–220	18
Endrin	18.19	263–193	25	281–245	14
PCB-123	18.23	324–254	21	326–256	21
PCB-118	18.29	324–254	21	326–256	21
Endosulfan II	18.40	241–206	11	272–237	11
4,4´-DDD	18.44	235–165	17	235–199	21
PCB-114	18.48	324–254	21	326–256	21
2,4´-DDT	18.49	235–165	17	237–165	11
PCB-153	18.68	358–288	20	360–290	25
PCB-105	18.76	324–254	21	326–256	21
4,4´-DDT	19.14	235–165	17	235–199	17
PCB-138	19.18	358–288	20	360–290	25
Tebuconazole	19.36	250–125	15	252–127	11
PCB-126	19.39	324–254	21	326–256	21
Triphenyl phosphate	19.42	326–233	11	325–169	15
Piperonyl butoxide	19.43	176–103	21	176–117	21
PCB-167	19.70	358–288	20	360–290	25
Phosmet	19.99	160–77	17	160–133	17
PCB-156	20.11	358–288	20	360–290	25
Benzo[a]anthracene	20.16	228–226	35	228–200	40
PCB-157	20.20	358–288	20	360–290	25
Chrysene	20.24	228–226	35	228–200	40
PCB-180	20.39	392–322	20	394–324	20
Pyriproxyfen	20.77	136–96	5	136–78	20
PCB-169	20.80	358–288	20	360–290	25
PCB-189	21.44	392–322	20	394–324	20
Coumaphos	22.00	226–163	17	362–334	13
Benzo[b]fluoranthene	22.86	252–250	40	252–224	40
Benzo[k]fluoranthene	22.93	252–250	40	252–224	40
Cypermethrin I	22.89	181–152	25	163–127	5
Cypermethrin II	23.04	181–152	25	163–127	5
Cypermethrin III	23.16	181–152	25	163–127	5
Cypermethrin IV	23.21	181–152	25	163–127	5
Benzo[a]pyrene	23.86	252–250	40	252–224	45
cis-τ-Fluvalinate	24.56	250–200	25	181–152	30
trans-τ-Fluvalinate	24.67	250–200	25	181–152	30
Indeno[1,2,3-cd]pyrene	28.54	276–274	40	276–248	50
Dibenzo[a,h]anthracene	28.73	278–276	35	278–252	35
Benzo[g,h,i]perylene	29.93	276–274	40	276–248	50

Note: RT, retention time; SRM 1 (Q), quantitative transition; SRM 2 (C), confirmation transition; CE, collision energy (eV); IS, internal standard.

Table 3. Validation parameters for contaminants analysed in Italian honey samples.

Compounds	Matrix effect, t_{cal}	LODs	LOQs	R^2	Recovery ± RSD (%)[a]			Intermediate precisions[a]	
					10 ng ml^{-1}	25 ng ml^{-1}	50 ng ml^{-1}	10 days	40 days
Acenaphtilene	0.562	0.12	0.40	0.9855	97 ± 12	102 ± 14	101 ± 8	2.3	6.1
Fluorene	0.236	0.10	0.33	0.9809	98 ± 10	103 ± 9	105 ± 6	2.0	5.5
α-HCH	3.654	0.12	0.40	0.9952	95 ± 9	97 ± 7	96 ± 3	3.6	6.6
Atrazine	0.123	1.11	3.66	0.9939	92 ± 11	94 ± 9	93 ± 7	4.8	7.5
β-HCH	0.032	0.11	0.36	0.9999	98 ± 8	101 ± 7	96 ± 4	2.1	4.6
γ-HCH	2.025	0.13	0.43	0.9949	99 ± 9	98 ± 10	99 ± 3	3.4	5.0
Diazinon	3.147	0.12	0.40	0.9967	91 ± 11	89 ± 9	87 ± 6	5.9	7.1
Phenanthrene	2.155	0.34	1.12	0.9889	100 ± 12	98 ± 10	98 ± 7	5.2	6.2
Anthracene	1.256	0.32	1.06	0.9831	102 ± 11	104 ± 4	107 ± 6	4.9	6.9
PCB-28	0.025	0.10	0.33	0.9913	94 ± 9	91 ± 6	92 ± 3	3.2	5.7
Alachlor	3.142	0.11	0.36	0.9898	93 ± 8	97 ± 5	100 ± 5	2.0	4.2
PCB-52	2.555	0.10	0.33	0.9897	102 ± 10	98 ± 2	99 ± 4	3.3	5.6
Chlorpyriphos-ethyl	2.684	1.00	3.30	0.9946	98 ± 8	102 ± 5	107 ± 4	2.2	4.9
Aldrin	1.965	1.31	4.32	0.9961	97 ± 11	99 ± 9	104 ± 6	4.8	6.2
cis-Chlorfenvinphos	2.654	0.32	1.06	0.9974	105 ± 13	99 ± 11	100 ± 8	6.0	8.3
trans-Chlorfenvinphos	2.895	0.15	0.49	0.9972	103 ± 7	100 ± 4	102 ± 3	5.9	8.0
cis-chlordane	2.698	0.12	0.40	0.9831	102 ± 8	100 ± 6	98 ± 2	3.1	6.8
2,4´-DDE	1.023	0.11	0.36	0.9974	96 ± 7	99 ± 7	97 ± 5	4.7	5.8
PCB-101	1.254	0.10	0.33	0.9943	104 ± 6	98 ± 5	100 ± 3	2.1	4.2
trans-Chlordane	0.950	0.13	0.43	0.9979	103 ± 8	101 ± 6	97 ± 4	2.6	4.7
Pyrene	0.568	0.12	0.40	0.9909	112 ± 16	102 ± 11	105 ± 7	3.0	5.0
4,4´-DDE	0.685	0.11	0.36	0.9983	105 ± 12	101 ± 8	99 ± 6	4.1	6.6
PCB-81	3.123	0.10	0.33	0.9937	96 ± 9	102 ± 6	98 ± 4	5.5	5.9
Bupirimate	1.321	1.42	4.69	0.9922	89 ± 18	94 ± 13	92 ± 9	6.2	8.7
Flusilazole	2.569	0.16	0.53	0.9893	94 ± 15	96 ± 11	97 ± 7	4.8	6.1
Buprofezin	2.789	2.55	8.41	0.9961	82 ± 17	84 ± 13	80 ± 15	4.4	5.6
2,4´-DDD	1.258	0.12	0.40	0.9948	98 ± 13	97 ± 9	95 ± 9	5.0	6.4
Dieldrin	0.685	2.57	8.48	0.9863	102 ± 12	99 ± 6	100 ± 5	2.0	4.0
PCB-77	2.403	0.10	0.33	0.9962	95 ± 8	99 ± 5	96 ± 3	3.9	7.1
Endrin	2.781	2.51	8.28	0.9942	99 ± 12	102 ± 9	105 ± 6	3.0	5.9
PCB-123	0.659	0.11	0.36	0.9992	94 ± 8	98 ± 5	101 ± 3	2.9	6.0
PCB-118	1.753	0.10	0.33	0.9955	94 ± 9	96 ± 7	94 ± 3	2.2	5.9
Endosulfan II	1.369	0.17	0.56	0.9902	85 ± 16	89 ± 12	87 ± 9	1.8	5.0
4,4´-DDD	2.147	0.13	0.43	0.9931	103 ± 13	100 ± 7	99 ± 8	1.6	4.8
2,4´-DDT	2.668	0.12	0.40	0.9890	88 ± 19	93 ± 13	92 ± 11	1.6	4.9
PCB-114	3.852	0.10	0.33	0.9956	97 ± 8	96 ± 5	97 ± 2	2.0	5.8
PCB-153	0.475	0.11	0.36	0.9950	95 ± 10	96 ± 6	101 ± 3	2.2	4.9
PCB-105	0.749	0.11	0.36	0.9980	99 ± 7	98 ± 5	98 ± 3	3.1	5.2
4,4´-DDT	1.259	0.15	0.49	0.9833	102 ± 20	104 ± 16	110 ± 15	3.6	5.8
PCB-138	1.542	0.11	0.36	0.9953	99 ± 8	95 ± 5	107 ± 2	4.6	6.1
PCB-126	1.721	0.10	0.33	0.9937	95 ± 9	96 ± 4	99 ± 2	4.2	5.9
Tebuconazole	0.987	1.21	3.99	0.9893	110 ± 16	105 ± 12	102 ± 10	1.7	3.9
Triphenyl phosphate	2.452	0.17	0.56	0.9983	101 ± 11	104 ± 8	109 ± 5	2.6	4.6
Piperonyl butoxide	2.642	0.33	1.09	0.9977	118 ± 8	109 ± 5	114 ± 3	3.0	5.1
PCB-167	2.861	0.12	0.40	0.9995	96 ± 8	99 ± 3	104 ± 3	3.3	5.9
Phosmet	0.589	0.36	1.19	0.9857	97 ± 11	98 ± 9	99 ± 7	4.1	7.7
PCB-156	0.721	0.12	0.40	0.9972	94 ± 7	96 ± 4	102 ± 2	3.9	6.8
Benzo[a]anthracene	1.942	0.14	0.46	0.9982	100 ± 13	102 ± 9	102 ± 7	4.3	7.6
PCB-157	2.016	0.12	0.40	0.9879	102 ± 11	100 ± 7	102 ± 3	1.6	4.0
Chrysene	3.276	0.36	1.19	0.9952	94 ± 8	98 ± 5	100 ± 6	1.9	4.1
PCB-180	3.571	0.10	0.33	0.9949	97 ± 10	99 ± 6	106 ± 2	3.2	5.6
Pyriproxyfen	0.350	0.12	0.40	0.9918	94 ± 13	102 ± 10	107 ± 8	4.0	6.1
PCB-169	2.543	0.11	0.36	0.9957	102 ± 11	99 ± 5	105 ± 3	2.4	4.2
PCB-189	2.753	0.10	0.33	0.9986	103 ± 9	108 ± 7	111 ± 4	1.1	3.8
Coumaphos	1.254	0.14	0.46	0.9913	99 ± 10	103 ± 8	113 ± 5	2.3	4.4
Benzo[b]fluoranthene	1.721	2.54	8.38	0.9960	102 ± 13	107 ± 10	110 ± 7	3.1	5.5
Benzo[k]fluoranthene	1.647	2.54	8.38	0.9938	90 ± 16	93 ± 11	91 ± 5	2.9	5.3
Cypermethrin I	2.352	5.10	16.83	0.9918	128 ± 18	135 ± 16	137 ± 13	2.7	4.8
Cypermethrin II	2.174	5.21	17.19	0.9982	125 ± 16	127 ± 17	125 ± 11	2.4	5.9
Cypermethrin III	2.641	5.17	17.06	0.9868	126 ± 18	130 ± 16	133 ± 10	2.0	4.9
Cypermethrin IV	2.658	5.14	16.96	0.9904	103 ± 11	99 ± 12	100 ± 8	4.5	6.0
Benzo[a]pyrene	0.721	0.34	1.12	0.9941	112 ± 16	105 ± 11	110 ± 8	6.0	8.1
cis-τ-Fluvalinate	3.993	2.52	8.32	0.9961	109 ± 13	107 ± 6	109 ± 7	5.5	7.3
trans-τ-Fluvalinate	3.821	2.52	8.32	0.9895	98 ± 15	90 ± 8	85 ± 10	6.2	8.3
Indeno[1,2,3-cd]pyrene	2.840	0.36	1.19	0.9847	125 ± 18	102 ± 13	112 ± 12	5.9	7.0
Dibenzo[a,h]anthracene	1.978	5.20	17.16	0.9857	115 ± 13	108 ± 11	116 ± 10	4.0	5.1
Benzo[g,h,i]perylene	1.773	0.37	1.22	0.9843	109 ± 15	115 ± 13	113 ± 13	4.1	6.2

Note: LODs, limits of detection (ng g^{-1}); LOQs, limits of quantification (ng g^{-1}); R^2, linear regression coefficient; RSD, relative standard deviation.
[a]Five replicates.

Table 4. Contaminant residues in Italian honey samples (each sample was analysed in triplicate).

Compounds	Contaminated samples (%)	Range of concentrations (ng g^{-1})	Mean concentration ± SD (ng g^{-1})[a]
Organophosphorus pesticides (OPPs)			
Chlorfenvinphos (*cis* + *trans*)	100	0.512–64.77	19.67 ± 20.21
Diazinon	30	n.d.–4.90	1.40 ± 1.12
Chlorpyriphos-ethyl	2	n.d.–3.05	–
Coumaphos	98	n.d.–145.5	15.52 ± 22.28
Phosmet	23	n.d.–5.72	2.42 ± 1.36
Triphenyl phosphate	85	n.d.–201.6	9.50 ± 32.83
Organochlorine pesticides (OCPs)			
2,4´-DDE	9	n.d.–0.371	0.351 ± 0.01
2,4´-DDD	9	n.d.–0.796	0.768 ± 0.02
4,4´-DDD	11	n.d.–1.16	1.15 ± 0.01
2,4´-DDT	13	n.d.–1.83	1.10 ± 0.56
4,4´-DDT	23	n.d.–1.49	1.06 ± 0.23
γ-HCH	19	n.d.–14.97	3.87 ± 4.29
Endosulfan II	13	n.d.–5.17	1.42 ± 1.84
Polycyclic aromatic hydrocarbons (PAHs)			
Acenaphtilene	2	n.d.–6.95	–
Fluorene	26	n.d.–7.70	1.77 ± 1.87
Phenanthrene	34	n.d.–5.83	2.13 ± 1.10
Pyrene	11	n.d.–3.67	2.20 ± 0.95
Fungicide (F), pyrethroid insecticide (PYR), synergist (SYN), insect growth regulator (IGR)			
Flusilazole	6	n.d.–1.06	0.65 ± 0.37
Tebuconazole	11	n.d.–53.39	13.64 ± 22.23
Piperonyl butoxide	28	n.d.–7.17	3.22 ± 1.76
τ-Fluvalinate (*cis* + *trans*)	2	n.d.–21.20	–
Pyriproxyfen	6	0.406–2.03	0.948 ± 0.001

Note: [a]Calculated only on contaminated samples; SD, standard deviation; n.d., not detectable (< LODs).