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Determination of Pesticides and Persistent Organic Pollutants in Honey by Accelerated Solvent Extraction and GC-MS/MS

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INTRODUCTION

Honey is a natural product that is widely used for both nutritional and medicinal purposes. It is generally considered a natural and healthy product of animal origin, free of impurities. However, honeybees are subject to a number of viral, bacterial, fungal, and parasitic diseases and infestations. Insecticides, fungicides, and acaricides are used to protect colonies against infestations from hive beetle and parasites. Many pollutants in the environment can also contaminate the bees themselves in addition to their pollen, honey, and other bee products. Pollutants such as organochlorine pesticides (OCs), polychlorobiphenyls (PCBs), organophosphates (OPs), and polybromodiphenylethers (PBDEs) are a particular threat due to their environmental persistence and ability to bioaccumulate in the food chain. Due to the potential toxicity, a comprehensive workflow method for the extraction and analysis of these environmental pollutants is of growing importance to ensure the health and safety of bees and their honey.

Among the available extraction techniques, accelerated solvent extraction (ASE) offers shorter extraction times and reduced solvent consumption. ASE uses high temperatures combined with high pressure. A high temperature allows a higher rate of extraction due to a reduction in viscosity and surface tension, and increases the solubility and diffusion rate into the sample. The method reported here is applicable for the extraction and analysis of four different classes of compounds (6 PCBs, 7 PBDEs, 16 OCs, and 19 OPs) in honey using ASE and GC-MS/MS.

MATERIALS AND METHODS

Sample Collection and Preparation

Beekeepers from three different Italian regions: Calabria, South Italy (14 samples); Trentino Alto Adige, North Italy (18 samples) and Lombardia, North Italy (27 samples) provided 59 organic honey samples, as summarized in **Table 1**. All samples were stored at -20 °C prior to analysis to prevent matrix decomposition.

Table 1. Areas of Origin for Organic Honey Samples.

Number of Samples	Area of Origin	Botanical Source	Potential Environmental Contamination Sources
27	Lombardia (North of Italy)	Multifloral	Industrialized Area (OCPs, PCBs, PBDEs)
14	Trentino (North of Italy)	Multifloral	Intensive Apple Orchard (Pesticides)
18	Calabria (South of Italy)	Citrus (Monofloral)	Intensive Citrus Orchard (Pesticides)

Working solutions were prepared by diluting the stock solution in hexane for pesticides and then stored at -40 °C. Mixed compound calibration solution, in hexane, was prepared daily from the stock solutions (10 µg/mL) and the appropriate volume was used as a spiking solution.

The extractions were carried out using a Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor shown in **Figure 1**, equipped with 34 mL stainless steel extraction cells. The extracts were collected in 60 mL vials, treated with sodium sulfate and directly concentrated in a 2 mL autosampler glass vial using a Thermo Scientific™ Rocket™ Evaporator system (**Figure 1**).

Figure 1. Dionex ASE 350 Accelerated Solvent Extractor and Rocket Evaporator.

Dionex ASE 350 Accelerated Solvent Extractor

Rocket Evaporator System

A cellulose filter was placed in the bottom of a 34 mL extraction cell (**Figure 2),** followed by 5 g of activated Florisil and another cellulose filter. A 2 g sample of honey was homogenized with an equal weight of Thermo Scientific™ Dionex™ ASE™ Prep DE dispersant, sodium sulfate and transferred into the cell. One mL of isooctane solution containing the three internal standards was added. The remaining empty volume was filled with Dionex ASE Prep DE dispersant. The extractor was programmed according to the conditions reported in **Table 2**. The extracts were collected in 60 mL vials and treated with sodium sulfate to remove any possible water. After filtration, the organic phase was concentrated to dryness using the Rocket Evaporator system, dissolved in 200 µL of isooctane, and submitted to analysis by GC-MS/MS.

Figure 2. Extraction Cell Schematic.

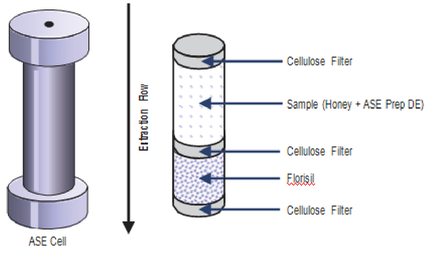


Table 2. Dionex ASE 350 Accelerated Solvent Extractor Extraction Method.

Parameter	Setting
Solvent	n-Hexane/Ethyl Acetate (4:1, v/v)
Temperature	80 °C
Pressure	1500 psi
Static Cycles	3
Static Cycle Time	3 min
Rinse Volume	90%
Purge Time	90 s
Extraction Time per Sample	~15 min
Solvent Used per Sample	~ 50 mL

Analytical Methods

The samples were analyzed using a Thermo Scientific™ TRACE™ 1310 gas chromatograph equipped split/splitless injector, a fused-silica capillary column (Rt-5MS Crossbond-5% diphenyl 95% dimethylpolysiloxane, 35 m × 0.25 mm × 0.25 µm,) and a Thermo Scientific™ TSQ™ 8000 triple quadrupole GC-MS/MS (**Figure 3**). The method conditions for the gas chromatograph and mass spectrometer are listed in **Tables 3 and 4**.

Figure 3. Thermo Scientific™ TSQ™ 8000 Triple Quadrupole GC-MS/MS.

Table 3. GC and Injector Conditions.

Parameter	Setting
Injector Type	PTV, Splitless
Injector Temperature	250 °C
Liner	2 x 2.75 x 120 mm
Injected Volume	1 µL
Splitless Time	0.5 min
Splitflow	20 mL/min
GC Column	Rt-5MS (35m x 0.25 mm x 0.25 µm)
Carrier Gas	Helium, 99.999% purity
Flow Rate	1.0 mL/min, constant
Initial Temperature	80 °C (3 min) 10 °C/min to 170 °C 3 °C/min to 190 °C 2 °C/min to 240 °C 3 °C/min to 280 °C 10 °C/min to 310 °C
Final Temperature	310 °C (5 min)

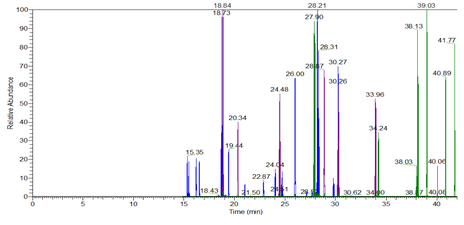
Table 4. Mass Spectrometer Parameters

Parameter	Setting
Source Temperature	270 °C
Ionization	EI
Electron Energy	70 eV
Emission Current	50 µA
Q2Gas Pressure	1.5 mTorr
Collision Energy	10 to 30 eV
Q1 Peak Width FWHM	0.7 Da
Q3 Peak Width FWHM	0.7 Da

RESULTS

A multiresidue method for the analysis of organic contaminants and pesticides was developed. The ASE extraction with inline cleanup was necessary for the removal of interfering substances (e.g., carbohydrates) from honey samples. Florisil proved to be very efficient for the cleanup of different foods as well as honey samples. The proposed method was optimized for the multiresidual analysis of 59 pesticides and persistent organic pollutants (POPs). Total ion current chromatograms (GC-MS/MS) of blank honey samples spiked with investigated compounds and a naturally contaminated sample are shown in **Figures 4 and 5**.

Figure 4. Total Ion Current (GC-MS/MS) Chromatogram of a Spiked Honey Sample (10 ng/g).

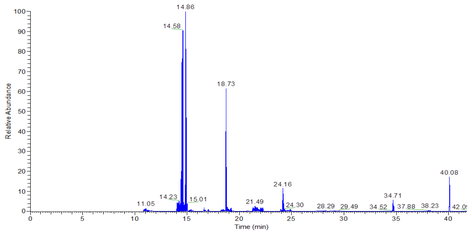


The method showed good linearity with determination coefficients equal to or higher than 0.99 for all of the compounds investigated. The method also showed good repeatability, demonstrating that it is effective for monitoring compounds belonging to different chemical classes (**Table 5**). The recoveries ranged from 97 to 102% for PCBs and PBDEs, from 75 to 95% for OCs, from 75 to 97% for OPs and from 75% to 102% for the additional agrochemicals. The CVs ranged from 4 to 14%. The one-step ASE method, using Florisil as an interference retainer, is rapid, cost-effective, and minimizes waste generation compared to the classic methods. Combining the extraction and the two clean-up steps (i.e., GPC and SPE) in a single ASE step reduced laboratory time by half. At present, this is the first ASE application using an inline cleanup step to screen for the presence of different pesticides and organic contaminants in honey.

Table 5. Recoveries (%, RSD), LOD, LOQ and coefficient of determination (r^2)

Contaminants	LOD (ng/g)	LOQ (ng/g)	Recovery % (RSD)	Coefficient of Determination (r^2)
Polychlorobiphenyls (PCBs)				
PCB 28	0.08	0.24	102 (7)	0.9994
PCB 52	0.07	0.21	103 (7)	0.9999
PCB 101	0.04	0.12	97 (4)	0.9999
PCB 138	0.05	0.15	105 (4)	0.9999
PCB 153	0.02	0.06	102 (4)	0.9999
PCB 180	0.06	0.18	98 (9)	0.9999
Polybrominated Diphenyl Ethers (PBDEs)				
PBDE 28	0.01	0.03	100 (9)	0.9991
PBDE 33	0.02	0.06	98 (9)	0.9999
PBDE 47	0.02	0.06	97 (8)	0.9996
PBDE 99	0.03	0.09	102 (7)	0.9998
PBDE 100	0.01	0.03	103 (7)	0.9998
PBDE 153	0.03	0.09	97 (10)	0.9992
PBDE 154	0.02	0.06	100 (12)	0.9999
Organochlorine Pesticides (OCPs)				
α-HCH	0.99	2.97	78 (10)	0.9959
Hexachlorobenzene	1.26	3.78	80 (12)	0.9945
β-HCH	1.17	3.51	85 (12)	0.9995
Lindane (γ-HCH)	0.79	2.39	96 (10)	0.9985
Heptachlor	0.95	2.84	93 (12)	0.9996
Aldrin	0.85	2.55	75 (14)	0.9991
Heptachlor Epoxide	0.91	2.73	77 (14)	0.9994
trans-Chlordane	1.48	4.44	92 (10)	0.9993
Endosulfan I	1.13	3.38	80 (13)	0.9992
pp'-DDE	0.85	2.55	97 (12)	0.9994
Endrin	0.99	2.98	88 (11)	0.9998
Endosulfan II	1.14	3.42	90 (10)	0.9993
pp'-DDD	0.91	2.74	87 (14)	0.9986
op-DDT	0.94	2.83	82 (14)	0.9963
Endosulfan Sulfate	1.07	3.22	85 (12)	0.9921
pp'-DDT	0.91	2.74	95 (12)	0.9992
Organophosphorus (OPs)				
Mevinphos	0.75	2.25	75 (12)	0.9996
Ethoprophos	0.44	1.32	86 (10)	0.9991
Dichlorvos	0.33	0.99	93 (10)	0.9997
Phorate	0.52	1.56	75 (13)	0.9993
Demephron (-O and –S)	1.12	3.36	77 (14)	0.9992
Diazinon	1.10	3.30	90 (10)	0.9994
Disulfoton	0.90	2.70	80 (14)	0.9998
Parathion-methyl	0.83	2.49	97 (8)	0.9993
Fenchlorphos	1.12	3.36	88 (11)	0.9986
Chlorpyrifos	0.95	2.85	90 (9)	0.9963
Fenthion	0.78	2.34	87 (12)	0.9996
Trichloronate	0.98	2.94	85 (14)	0.9998
Tetrachlorvinphos	1.12	3.36	85 (12)	0.9998
Prothiofos	0.75	2.25	92 (12)	0.9992
Terbuphos	0.68	2.04	90 (12)	0.9963
Fensulfothion	1.09	3.27	88 (10)	0.9921
Sulprofos	0.98	2.94	87 (10)	0.9992
Azinphos-methyl	1.07	3.21	87 (12)	0.9978
Coumaphos	0.78	2.34	84 (14)	0.9962

Figure 5. Total Ion Current (GC-MS/MS) Chromatogram of a Raw Honey Sample.



CONCLUSIONS

An analytical method was developed and successfully applied to evaluate pesticides and POP residues in organic honey samples produced in three different Italian regions that are characterized by different contamination sources. The method proved to be simple and rapid, requiring small sample sizes, and minimizing solvent consumption, due to the ASE with an inline cleanup step. MS/MS detection provided both quantitative information and the confirmation of POP residues in honey, confirming the one-step ASE method as a valid alternative to classical extraction methods.

REFERENCES

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TRADEMARKS/LICENSING

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