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Determination of Pesticide Residues in Honey Samples

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Abstract Protocol for the determination of pesticides residues in honey samples have been standardized using a simple technique of liquid–liquid extraction. The method is sensitive to detect low levels of pesticides in honey. Honey sample was fortified with pesticides, namely, cypermethrin, fenvalerate, alphamethrin, lamba–cyhalothrin, endosulfan (α, β and sulfate) and chlorpyrifos. The method of extraction and clean up was optimized and validated in the laboratory. The method was applied to screen six samples of honey locally available for pesticides residues. Recoveries ranged from 60% to 90.6% with RSDs from 2% to 10%. Low recoveries were recorded for α and β -endosulfan in the range of 60%–71%. The LOQs, varied from 0.05 to 1.0 mg kg^{-1}.

Keywords Honey · Pesticides residue analysis · GLC

Pesticides play a beneficial role in agriculture, because they help to combat the variety of pest that destroy crops, even though small amounts of pesticide residues remain in the food supply, constituting a potential risk for the human health, because of their sub-acute and chronic toxicity. The most widely used pesticides are organophosphorus and carbamates, which have almost completely replaced organochlorine pesticides. The extensive distribution of these groups of pesticides causes bees that have been fed on contaminated blossom to transfer pesticide residues into honey and finally to the consumer. Organochlorine pesticides, like DDT and BHC have been restricted or banned in India for use in agriculture since 1987–88 because of their persistence and bioaccumulation in the environment. However, DDT is still used in malaria eradication programmes, and in human welfare and hygiene. These pesticides are still frequently found in soil, from which they continue to cycle through the environment, as soil is a potential source to the atmosphere by way of volatilization and to water, plants, and animals, by their movement via runoff. Different studies demonstrated the bioaccumulation of organochlorine from contaminated soil to aerial and root tissues of different plants and to organisms, which can bioconcentrate these fat-soluble pesticides at 10–1,000 times the level found in the surrounding environment. The presence of pesticide residues in honey has impelled the need for setting up monitoring programs to determine the proper assessment of human exposure to pesticides making possible to take policy decisions in the interest of health hazard. Different national regulations have established maximum concentrations of pesticide residues (MRLs) permitted in honey, but the lack of homogeneity causes problems in international marketing and trade. As an example, Germany, Italy, and Switzerland have set MRLs (Rissatoa et al. 2007) for amitraz, bromopropylate, coumaphos, cyamizole, flumetrine, and fluvalinate, which oscillate between 0.01 and 0.1 mg kg^{-1} in Germany, between 5 and 500 mg kg^{-1} in Switzerland, and are of 10 mg kg^{-1} in Italy. Up to now, maximum limits of pesticide residues in honey are not included in the *Codex Alimentarius* (1998). The European Union (EU 1996, 1999) legislation has regulated the MRLs for three acaricides: amitraz, coumaphos, and cyamizole, which are 0.2, 0.1, and 1 mg kg^{-1}, respectively (EC 1990). The U. S. Environmental Protection Agency (FDA, USA 2003) has established MRLs for amitraz (1 mg kg^{-1}), coumaphos (0.1 mg kg^{-1}), and fluvalinate (0.05 mg kg^{-1}).

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Previous report of the study performed in 27 honey samples from India from 1993 to 1995 showed that all samples were contaminated by organophosphorus, mainly DDVP, chlorpyriphos, monocrotophos, dimethoate, and fenitrothion (Rathi et al. 1997). Carbofuran and carbaryl contaminated 55% of the honey samples. Blasco et al. (2003) reported that the honey samples from Portugal and Spain contained mostly organochlorine along with other insecticides. All honey samples studied were also contaminated with organochlorines, but the amount of residues found was much lower than that of organophosphorus and carbamates.

A multiresidue method which is able to detect as many pesticides as possible, in a relatively short time period, is crucial for an efficient monitoring program. Generally, these methods are based on the traditional liquid–liquid extraction (LLE; Deka et al. 2004) or solid-phase extraction (SPE; Campillo et al. 2006; Fernández Muíño and Simal Lozano 1991), or by ultrasonic extraction method (Rezić et al. 2007). The main advantage of LLE is simplicity but employs a large amount of toxic solvent and is a time-consuming procedure. Much less toxic solvents are consumed by SPE, which also offers a save in sample preparation time. However, this technique has the disadvantage of being unable to handle large sample volumes. Both, LLE and SPE have been selected in various multiresidue methods for extracting organochlorine, organophosphorus, carbamate, and pyrethroid pesticides in honey. The detection of pesticides is accomplished by gas chromatography (GC) or liquid chromatography (LC). Until now, GC has been the most widely used technique, because it's high separation power and availability of selective detectors. This paper presents a simple protocol for determination of pesticides in honey samples.

Materials and Methods

All the chemicals were obtained from SD Fine Chem Ltd. Mumbai, India. *Solvents*: acetone, hexane, ethyl acetate and dichloromethane; *Adsorbents*: SPE silica cartridge RANKEM, Florisil. *Drying agent*: anhydrous sodium sulfate. The solvents were distilled before use. An individual stock solution of each pesticide chlorpyrifos, chlorthalonil, α-endosulfan, β-endosulfan, endosulfan sulfate, α-methrin, λ-cyhalothrin, cypermethrin, and fenvalerate was prepared separately. A mixture of 10 µg in 10 mL of the mentioned pesticides was prepared and the volume made up to the mark with hexane. This stock solution of mixture was diluted in hexane to prepare at 1 µg mL^{-1} and stored at 4°C. Working standards were prepared by appropriate dilutions. The recovery study were carried out by spiking the honey sample (5 g) in triplicate with the pesticide mixture, the fortification level for fenvalerate and endosulfan sulfate was 1 µg; α-endosulfan-β-endosulfan, λ-cyhalothrin, alphamethrin, and cypermethrin were spiked at 0.5 µg level and chlorpyrifos at 0.05 µg level.

The method of extraction and clean up followed is as described. Honey samples of different brands were collected from local markets of Delhi. Representative honey sample (5 g) in quadruplicate was diluted with 4% sodium sulfate solution (100 mL) and subjected to liquid–liquid partitioning thrice with ethyl acetate (50, 30, 30 mL). The ethyl acetate extract was centrifuged at 2,000 rpm for 2 min to break the emulsion (formed after shaking with ethyl acetate). The organic phase was filtered through 5 cm layer of anhydrous sodium sulfate and the filtrate was concentrated and passed through a SPE silica cartridge. The cartridge was conditioned with hexane and 6 mL of the concentrated extract loaded on to it. The cartridge was eluted with 50 mL of 15% diethyl ether in hexane. The cleaned extract was evaporated under vacuum and was analysed by GLC using EC detector. The pesticides of concern were chlorpyrifos, endosulfan, alphamethrin, cypermethrin, fenvalerate and deltamethrin.

Gas Chromatograph (Hewlett Packard Model 5890 Series II) equipped with ^{63}Ni electron capture detector and HP-1 column (25 m × 0.53 mm × 1 μ) the operating conditions were 170°C hold @ 3°C 220 hold min @ 270°C hold 10 min. The injector and detector temperatures were 270 and 300°C, respectively. The carrier gas, nitrogen flow was maintained at 2 mL/min (Fig. 1).

Alternate column packing was used to confirm the pesticides detected. The analysis was carried out on Varian GLC CP-3800 fitted with capillary column WCOT fused silica column CP-SIL 5CB (30 m × 0.25 mm × 0.25 μ). The oven temperature was programmed 170°C hold for 2 min @ 3°C/min to 220°C 20°C/min @ 270°C hold 15 min. The injector and detector temperatures were 270 and 300°C, respectively. The total run time was 25.33 min. The carrier gas flow was 2 mL/min and make up flow was 27 mL/min. The retention time of pesticides are given in the Table 1.

Results and Discussion

Recovery experiments were carried out by spiking honey samples (5 g) with the pesticide mixture at appropriate concentrations in methanol. The precision and accuracy of the procedure was carried out by analysis of 4-spiked honey samples at two concentration levels (the limits of quantification (LOQs) and five times the LOQ).

Recoveries ranged from 60 to 90.6% with RSDs from 2 to 10% (Table 2). Low recoveries were recorded for α and β -endosulfan in the range of 60–71%. The LOQs, varied from 0.05 to 1.0 mg kg^{-1}. The LOQs, varied from 0.05 to

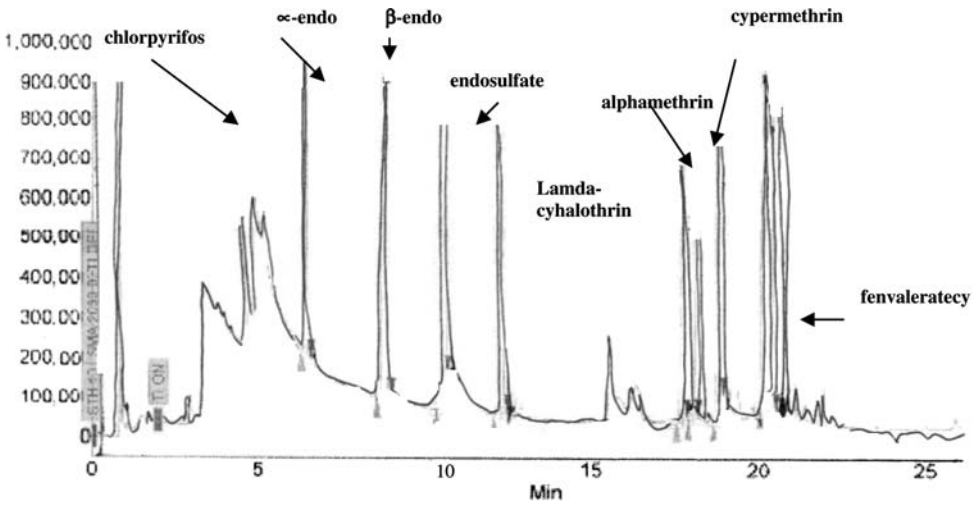

Fig. 1 Gas chromatogram profile of pesticides mixture

Table 1 Retention time and IDL and LOQ

S. no.	Pesticide	Retention time	Instrument detection limit (μg)	Limit of quantitation (μg)
1	Chlorpyrifos	6.43	0.001	0.05
2	α-Endosulfan-	8.71	0.01	0.5
3	β-Endosulfan	10.45	0.01	0.5
4	Endosulfan sulfate	12.23	0.05	1.0
5	λ-Cyhalothrin	17.97	0.01	0.5
6	Alphamethrin	17.68	0.01	0.5
7	Cypermethrin	18.74	0.01	0.5
8	Fenvalerate	20.43	0.05	1.0

Table 2 Percent recovery of pesticides from honey

S. no.	Pesticide	Amount fortified (μg)	Amount recovered (μg) (SD)	% RSD[a]	% Recovery (SD)	% RSD
1	Chlorpyrifos	0.05	0.042 (0.006)	14.44	80.0 (8.71)	10.89
2	α-Endosulfan-	0.5	0.356 (0.037)	10.61	71.3 (7.57)	10.67
3	β-Endosulfan	0.5	0.30 (0.010)	3.33	60.0 (2.00)	3.33
4	Endosulfan sulfate	1.0	0.78 (0.036)	4.62	78.0 (3.60)	4.62
5	λ-Cyhalothrin	0.5	0.453 (0.015)	3.36	90.6 (3.05)	3.36
6	Alphamethrin	0.5	0.373 (0.015)	4.09	74.6 (3.05)	4.09
7	Cypermethrin	0.5	0.42 (0.01)	2.38	84.0 (2.00)	2.38
8	Fenvalerate	1.0	0.79 (0.041)	5.24	79.3 (4.16)	5.24

[a] n = 3

0.1 μg/g. These values correspond to the lowest concentration of compound that gives a response that can be quantified. The detection of organochlorine pesticides was performed by capillary gas chromatography with electron-capture detection. The limit of detection (LOD) was less than10.0 μg/kg for all the pesticides except for chlorpyrifos which LOD was 1 μg/kg. The developed method was linear in the range of 10–500 μg/kg, with correlation coefficients larger than 0.995. Finally, the developed analytical method has been successfully applied to the determination of pesticide residues in several honey samples (Table 3).

Similar results have been reported by Choudhary and Sharma (2008). Among the pesticides analysed in honey in Himachal Pradesh, India, the most frequently detected was HCH and its isomers, followed by DDT isomers. Among

Table 3 Monitoring of pesticide residues (mg/kg) in honey

Insecticide	Brand I	Brand II	Brand III	Brand IV	Brand V	Brand VI	Mean residues (mg/kg) (range)[a]
Chlorpyrifos	0.02	0.02	0.21	ND	ND	ND	0.05 (ND–0.21)
α-Endosulfan-	ND	0.22	ND	0.22	0.41	ND	0.17 (ND–0.41)
β-Endosulfan	0.18	0.33	0.12	0.15	0.31	ND	0.22 (0.12–0.33)
Endosulfan sulfate	ND	ND	ND	0.14	0.41	ND	0.11 (ND–0.41)
Alphamethrin	ND	ND	0.04	ND	ND	ND	0.07 (ND–0.34)
Cypermethrin	0.005	ND	ND	ND	ND	ND	0.005 (ND–0.005)
Fenvalerate	0.01	ND	ND	ND	ND	ND	0.01 (ND–0.01)

[a] $n = 4$

the synthetic pyrethroids, only cypermethrin was found in honey samples. Residues of organophosphates viz. acephate, chlorpyriphos, ethion and monocrotophos were not detected; however, residues of malathion were found exceeding the MRL (5 ppb) as proposed by the Ministry of Commerce, Government of India. It is significant to mention that honey obtained from natural vegetation contained lesser residues. It can be concluded that honey from Himachal Pradesh had low pesticide residues.

Honey, being a natural product manufactured by honey bees is considered to be free from any extraneous material. The over-reliance on pesticides caused several environmental problems including pesticide residues in food. This constitutes a potential risk for human health, because of their sub acute and chronic toxicity. Therefore, it is imperative to monitor the presence of pesticide residues in honey, to know the extent of pesticide residue present in honey.

The method is reliable and can be considered useful for routine monitoring. None of the honey samples analyzed contained the studied compounds at concentrations above the corresponding detection limits.

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