Determination of organochlorine pesticide residues in honey, applying solid phase extraction with RP-C18 material†

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In this study, a new clean up method was developed for the routine multiresidue determination of organochlorine pesticide residues in honey. The analytical procedure requires sample extraction with methanol, followed by a clean up step through a C18 Sep-Pak cartridge. Finally, pesticides are eluted with hexane. The determination of organochlorine pesticide residues was performed by capillary gas chromatography with electron capture detection. The mean recoveries of 18 organochlorine pesticides were estimated at various concentrations and found very efficient in most cases. The detection limits were found to be between 0.05 and 0.20 μg kg^{-1}.

The occurrence of organochlorine compounds in the food chain has already been reported in several studies.[1–4] This class of organic compounds consists of one of the most important groups of dangerous organic contaminants.

Honey is an exported product of Greece with great economic importance. According to EEC regulations, honey as a natural product, must be free of any chemical contaminants and safe for human consumption.[5] Many methods have been reported for the determination of pesticides in honey, used against the Varroa mite diseases (acaricides and organophosphorous pesticides)[6–11] or in agriculture for insect control on numerous field crops.[12,13] However, only a few are concerned with organochlorine pesticides although their occurrence has been reported in several studies.[14–17] These last methods, following the classical analytical procedures for the determination of pesticides in non-fatty foods, employ time consuming clean-up steps that make them impractical for routine analysis. It is therefore necessary for monitoring purposes to develop a specific and rapid method for the determination of organochlorine pesticide residues in this substrate.

Fernandez Muino and Simal Lozano[18] proposed a multiresidue method for determination of organochlorine pesticides in honey, which uses a Florisil clean up step for the isolation of pesticides followed by gas chromatography with electron capture detection (GC-ECD). Good recoveries of eight organochlorine pesticides were obtained together with a minimized matrix interference. However this method involves a complicated liquid–liquid extraction step in which there is a possibility of formation of a whitish gel which obscures the separation and gives recoveries below 60%. Furthermore, the proposed method was applied only to a small group of organochlorine pesticides.

In the present study a quick and simple alternative method, by drastically reducing the liquid–liquid extraction step, for the determination of 18 organochlorine pesticides in honey, is presented. This method involves sample extraction with methanol, followed by solid phase extraction on C18 cartridges and elution with hexane.

The target compounds studied, namely α-HCH, β-HCH, lindane, δ-HCH, heptachlor, aldrin, heptachlor epoxide, α-endosulfan, 4,4-DDE, dieldrin, endrin, β-endosulfan, 4,4-DDD, endrin aldehyde, endosulfan sulfate, 4,4-DDT, methoxychlor and endrin ketone, were determined by capillary gas chromatography with electron capture detection (GC-ECD). Confirmation was achieved using two GC columns of different polarity.

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Experimental

Materials

The solvents used (methanol and hexane) were pesticide residue free (Pestiscan, Lab Scan, Dublin, Ireland). Water was the product of Reidel-de Haen (Pestanal), Seelze, Germany. α-HCH and endrin were obtained as solid materials from Reidel-de Haen, with a purity of 98–99%. Lindane and aldrin were obtained as solid materials from Alltech, Chicago, IL, USA, with purities of 99%. The other pesticides were obtained from Polyscience, Niles, IL, USA, as solutions in methanol. Stock solutions of each pesticide were prepared in methanol at 1000 μg ml^{-1}. The mixture of the 18 organochlorine pesticides were purchased from Polyscience, as a solution of 2000 μg ml^{-1} in methanol. Working solutions were prepared by diluting the stock solutions as required. Solid phase extraction was carried out using bonded-phase silica C18 0.85 ml filled cartridges, containing 360 mg of C18 octadecyl sorbent, Sep-Pak 'classic', product of Waters, Milford, MA, USA.

Procedure

Sample extraction and clean up. Honey (10 g) was dissolved in 50 ml of methanol and the mixture was stirred for an hour. Then 25 ml of the above solution, after filtration, was diluted in 2 l of distilled water, at pH 2. The mixture was passed through a C18 cartridge, which had previously been conditioned with 10 ml of methanol and then with 5 ml of water. The C18 cartridge was fitted to a glass column (25 cm × 1 cm) which was connected to a 2 l flask reservoir. Head pressure was applied with extra pure nitrogen to increase the flow, to about 10 ml min^{-1}. After the sample volume had passed through the column, the cartridge was dried for 1 h under a stream of

nitrogen and the organochlorine pesticides were eluted with 10 ml of hexane. The extract was rotary evaporated (bath temperature, 30 °C) to ≈1 ml and the residue transferred quantitatively with hexane into a 5 ml volumetric flask for the GC-ECD analysis.

Gas chromatographic analysis. The analysis of the 18 organochlorine pesticides was carried out by capillary gas chromatography using the following instruments: (1) Varian (Paolo Alto, CA, USA) Model 3400 gas chromatograph equipped with ECD, split/splitless injection port, a DB-1 fused silica capillary column by J&W Scientific, Rancho Cordova, CA, USA (30 m × 0.32 mm id, 0.25 μm film thickness) and autosampler Model 8200 cx, with a program for the evaluation of GC runs (DAPA Scientific, Kalamunda, Australia); and (2) Carlo Erba (Milan, Italy) Model Mega 2 gas chromatograph equipped with ECD, split/splitless injection port, a DB-5 fused silica capillary column by J&W Scientific (30 m × 0.25 mm id, 0.25 μm film thickness) and autosampler Model A200S, with a program for the evaluation of GC runs (Chrom-Card, Fisons Instruments, Rodano, Milan, Italy).

The temperature program applied was as follows: 80 °C for 1 min, 80–218 °C at 8 °C min^{-1}, 218 °C for 18 min, 218–250 °C at 4 °C min^{-1} and 250 °C for 10 min. The injection was carried out splitless at 250 °C and the injection volume was 1 μl.

Standard solutions of each target compound were analysed under the mentioned conditions on DB-1 and DB-5 columns for the determination of their retention times.

The linearity of the ECD system was tested by analysing standard solutions of the studied pesticides in the range 0.2 to 40 μg l^{-1}. Five point external standard calibration was used for the quantitative measurements.

Recovery experiments and detection limits. Recovery experiments, concerning the 18 organochlorine pesticides, were carried out, in triplicate, at various fortification levels, by adding known volumes of pesticide standards in hexane, to homogenized honey samples. After solvent evaporation the samples were analysed according to the proposed method.

The recovery values were calculated from calibration graphs that were constructed from the concentration and peak area of the chromatograms obtained with standards of the organochlorine pesticides. Blank analyses were performed in order to check interference from the sample.

The detection and quantification limits of the target compounds were determined after spiking honey samples at lower concentration levels. Their values were calculated considering a signal-to-noise ratio of 3 or 10, respectively.

Results

Retention times (t_R) of 18 organochlorine pesticides were determined individually on DB-1 and are presented in Table 1. The GC-ECD chromatogram of a honey sample, spiked to 0.4 μg l^{-1} for each organochlorine pesticide is presented in Fig. 1. The resolution of lindane/β-HCH and 4,4 DDE/dieldrin pairs was poor under the conditions employed in this study. Therefore, the identification of peak identity was performed on DB-5 column (t_R = 17.73/17.97 and 26.63/26.87, respectively).

The matrix interference during analysis of honey samples in the GC-ECD system was limited. Gas chromatograms of spiked honey samples were quite similar to those obtained with the standard solution of pure pesticides. For that reason, the preparation of standard solutions in control sample extracts was not necessary. The gas chromatogram of honey extract, presented in Fig. 2, shows good baseline stability with a few interfering peaks, indicating that the proposed clean up is suitable for the determination of the target analytes.

The detector response for all target compounds was linear in the concentration range 0.2 to 40 μg l^{-1} and the correlation

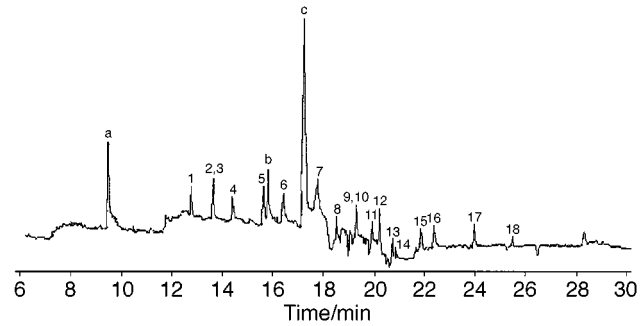

Fig. 1 Gas chromatogram of a honey sample extract spiked at 0.4 μg kg^{-1}, on a DB-1 column with ECD. The numbers refer to pesticides, according to Table 1; a, b and c are interfering peaks.

Table 1 Retention times (t_R), detection limits, mean percentage recoveries and relative standard deviations (RSD) of 18 organochlorine pesticides at three different fortification levels in honey (n = 3) on a DB-1 column (GC-ECD)

No.	Organochlorine pesticides	t_R/ min	Detection limit/ μg kg^{-1}	Mean percentage recovery (RSD)		
				20 μg kg^{-1}	10 μg kg^{-1}	4 μg kg^{-1}
1	α-HCH	12.67	0.08	66 (6)	71 (4)	80 (12)
2	β-HCH	13.57	0.06[a]	72 (5)	84 (6)	83 (8)
3	Lindane	13.57	0.08[a]	65 (8)	93 (8)	86 (10)
4	δ-HCH	14.22	0.08	120 (6)	85 (4)	107 (13)
5	Heptachlor	15.54	0.08	66 (3)	85 (10)	77 (3)
6	Aldrin	16.43	0.10	61 (5)	87 (7)	72 (2)
7	Heptachlor epoxide	17.44	0.09	75 (7)	82 (4)	81 (4)
8	α-Endosulfan	18.42	0.12	71 (4)	73 (1)	56 (6)
9	4,4-DDE	19.20	0.10[a]	80 (6)	77 (7)	79 (2)
10	Dieldrin	19.25	0.10[a]	83 (5)	103 (4)	80 (6)
11	Endrin	19.88	0.16	108 (3)	94 (3)	88 (5)
12	β-Endosulfan	20.18	0.05	72 (4)	74 (6)	87 (8)
13	4,4-DDD	20.70	0.16	116 (7)	101 (7)	77 (7)
14	Endrin aldehyde	20.87	0.20	80 (3)	104 (1)	48 (8)
15	Endosulfan sulfate	21.88	0.18	74 (2)	92 (4)	73 (4)
16	4,4-DDT	22.27	0.10	79 (4)	102 (2)	125 (2)
17	Methoxychlor	24.01	0.08	81 (3)	73 (3)	53 (3)
18	Endrin ketone	25.61	0.20	74 (2)	83 (4)	78 (4)

[a] Refers to the DB-5 column

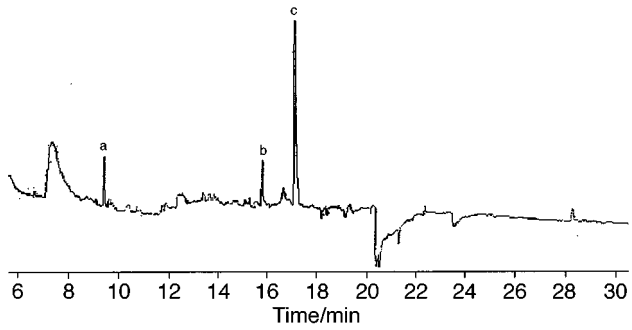

Fig. 2 Gas chromatogram of an unspiked honey sample extract, on a DB-1 column with ECD; a, b and c are interfering peaks.

coefficients were better than 0.999. The calculation of the amount of the organochlorine pesticides present was carried out using the DB-1 column. The results were confirmed with the DB-5 column. In case one or more of the pesticide pairs that were not resolved on the DB-1 column were present, identification was performed with the DB-5 column. Quantification was carried out by DB-5 only when both components of each pair were identified, otherwise it was based on DB-1.

Recovery experiments, concerning the 18 organochlorine pesticides, were performed in honey samples, at three fortification levels of 4, 10 and 20 μg kg^{-1}. The results of a series of threefold experiments for each fortification level are presented in Table 1. The mean recoveries, at the three fortification levels, approach successful recovery in most cases. The mean recoveries of honey samples fortified at the 20 μg kg^{-1} level were between 61 and 120%. The recoveries of the same pesticides at the 10 μg kg^{-1} level, ranged from 71 to 104%. The recoveries at the lower fortification level (4 μg kg^{-1}) were between 72 and 125% except for endrin aldehyde, methoxychlor and α-endosulfan which was only recovered with 48, 53 and 56%, respectively. It seems that the recovery values were not related to the spiking level. The precision of the method expressed by the relative standard deviation (RSD) of the mean recovery values, when triplicate spiked honey samples were analysed, was better than 13%.

The detection limits of the target compounds were in the range of 0.05 to 0.20 μg kg^{-1} and are shown in Table 1. The corresponding quantification limits, always 3.3 times the detection limits, varied between 0.16 and 0.66 μg kg^{-1} and were approximately four times lower than those reported in the literature.[18]

Conclusions

In this paper, a routine multiresidue method, for the determination of the 18 most important organochlorine pesticide residues in honey, is reported. This method applying solid phase extraction, followed by gas capillary chromatography with electron capture detection, is effective for the analysis of the target analytes and at the same time is quick and of low cost.

Solid phase extraction with RP-C18 material, without a further clean up step, yields high recovery rates for almost all compounds investigated.

The main advantages of the method described, compared to previously reported analytical procedures, are: (a) sample treatment is easier and faster; and (b) a larger number of organochlorine pesticides can be simultaneously determined. Furthermore, with the analytical method presented, trace level determination of organochlorine pesticides at sub-ppb levels is possible and gives reliable results.

The lack of interferences due to the complex matrix, the high recovery values, and the sensitivity of this method offer a valuable tool for the determination of organochlorine pesticides in honey samples.

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