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Determination of residues of endosulfan and five pyrethroid insecticides in virgin olive oil using gas chromatography with electron-capture detection

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Abstract

A simple, fast and economical method has been developed for the determination of endosulfan and five pyrethroid insecticides, cypermethrin, deltamethrin, fenvalerate, λ-cyhalothrin and permethrin, in virgin olive oil. The method uses a Sep-Pak alumina-N column cleanup after a liquid–liquid extraction or low-temperature precipitation step, and gas chromatography (GC) with electron-capture detection. The matrix effect was assessed for the GC systems used. Recoveries were 71–91% with RSD values of 6–17%. The method was applied to 338 virgin olive oil samples for monitoring of residues of these pesticides. Cypermethrin and λ-cyhalothrin were detected at the limit of quantification in one sample each, while 22% of samples contained endosulfan residues, mostly at very low levels ranging from 0.02 to 0.57 mg/kg.

Keywords: Olive oil; Food analysis; Endosulfan; Pyrethroids; Pesticides

1. Introduction

Olive trees are attacked by a variety of insects and other pests, which cause a reduction in the quality and quantity of the olives and oil produced. Many of these are controlled by organophosphorus pesticides (OPPs) and methods for the determination of residues of this class of compound may be found in the literature [1,2]. In addition, endosulfan and several synthetic pyrethroid pesticides, namely cypermethrin, deltamethrin, fenvalerate, λ-cyhalothrin, and permethrin, are registered for use in olive groves in Greece [3]. These compounds are lipophilic, with high *n*-octanol–water partition coefficients ($K_{o/w}$) ranging from 4.7 to 7.0 (log values), which suggests that residues will concentrate in the oil during extraction from olive fruits.

Although a considerable amount of work has been carried out on the determination of the organochlorine pesticides (OCPs) in fatty matrices [4,5], references to the analysis of the pyrethroids and endosulfan in edible oils are relatively few [6–9]. Cleanup using an alumina column gave a good recovery from vegetable oils for α-endosulfan but not for deltamethrin [6,9] and the use of a series of solid-matrix partition steps (diatomaceous earth, florisil, solid-matrix sulfuric acid treatment) did not

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allow endosulfan sulfate to be recovered from olive oil [8]. Gel permeation chromatography followed by determination using gas chromatography with mass spectrometry in the selected ion monitoring mode (GC–MS-SIM) would appear to give acceptable recoveries for endosulfan and pyrethroid pesticides from oil matrices [7,9]. However, for determination using electron-capture detection (ECD), a further cleanup step is generally required because of excessive interferences in the chromatogram from matrix components and the need to protect the chromatographic system from the effects (losses in efficiency and sensitivity) of traces of fatty materials. It has been estimated that a fat residue of less than 0.25 mg/ml is required for ECD analysis [10].

Studies in the literature show that the cleanest ECD chromatograms are generally obtained after cleanup with alumina, but that both pesticide and lipid retention are proportional to the alumina activity and that fat itself deactivates alumina in a similar way to its deactivation with water [6,10]. In addition to optimising the activity of the alumina for fat retention, it is also important to ensure that a small excess of fat-free alumina exists at the foot of the column [6]. This excess must be sufficient to adsorb any fat tending to move down the column during elution but not too large, or of too great an activity, to lead to a reduced recovery of pesticides through their retention on the column.

The purpose of this study was two-fold. Firstly, to develop and validate a simple, low-cost method suitable for the analysis of trace amounts of endosulfan and the pyrethroid pesticides in virgin olive oil, a matrix which is particularly prone to give interferences in the ECD chromatogram from lipids co-eluting from cleanup [10]. Secondly, to apply this method to virgin olive oil samples, analysed within the context of a monitoring programme, in order to make an initial assessment of the impact of residues of these pesticides in olive oil.

2. Experimental

2.1. Reagents and materials

All solvents were of pesticide residue analysis grade. Analytical standards, which were obtained from various suppliers, had certified purities ranging between 94.9 and 99.5%. The solid-phase extraction (SPE) cartridges tested contained 500 mg of adsorbent and included the following: Isolute florisil, silica gel and alumina-N (International Sorbent Technology, Hengoed, UK) and Sep-Pak alumina-N (Waters, Milford, MA, USA). In addition, microcolumns were prepared in the laboratory using 500 mg or 1 g alumina neutral Woelm (activity 1), deactivated by shaking with 5% water by mass until no lumps were present and left overnight in a tightly closed container.

2.2. Oil samples

Virgin olive oil samples from a national monitoring programme were used for this study and came from different producers, tree varieties and regions of the country. Three hundred and thirty-eight samples were analysed over a 3-year period, and those ascertained to contain undetectable residues of the pesticides under study were used as matrices for the fortified samples.

2.3. Gas chromatography

The GC determinations were carried out using Hewlett-Packard (Avondale, PA, USA) 5890 Series II and 6890 gas chromatographs with ECD systems operated at 300°C, Hewlett-Packard 7673 autosamplers and split–splitless injectors operated in the splitless mode (220°C, 60 s, 1 μl). The carrier and make-up gases were helium and nitrogen (50 ml/min), respectively. Four capillary columns were tested: HP-5MS, Rtx-5 (both 5% phenylmethylpolysiloxane) and Rtx-50 (50% phenylmethylpolysiloxane) columns with dimensions 30 m×0.250 mm I.D., 0.25 μm film thickness, and an HP608 column with dimensions 30 m×0.530 mm I.D., 0.5 μm film thickness. The HP-5MS column was used for screening for most of the samples and the Rtx-50 column for confirmation. The oven temperature programme used for all columns was: 80°C, hold 1 min; 15°C/min to 190°C; 3°C/min to 280°C, hold 15 min. Quantification was carried out using calibration curves with three to five levels which covered the appropriate concentration range (typically 0.002 to 0.05 μg/ml or less). Matrix-matched calibration

standards were used for all quantifications, although the influence of the oil matrix on recoveries was periodically assessed by injecting standards in acetonitrile. Confirmation of endosulfan sulfate residues with GC–MS was carried out using a Varian (Walnut Creek, PA, USA) Star 3600 CX gas chromatograph fitted with a HP-5MS capillary column of the same dimensions as that described above and coupled to a Varian Saturn 2000 ion trap mass spectrometer.

2.4. Extraction

Two initial extraction steps were used during this study, both of which gave an extract in acetone equivalent to 1 g oil/ml. The first, which was used routinely until recently in one of our laboratories for the determination of OPP residues in virgin olive oil samples, involves partitioning a 5 g sample of virgin olive oil between hexane and acetonitrile phases (liquid–liquid partitioning) [11]. The second, which has recently been developed, involves partitioning between 5 g of oil and acetonitrile followed by removal of the oil by precipitation at −20°C (low-temperature method) [12]. Liquid–liquid partitioning was used for the method development work, for the original method validation and for monitoring samples from the first 2 years of the study. However, since the low-temperature method proved to be considerably faster and more cost-effective, method validation was repeated with this as the initial extraction step. Both methods were also used for the later studies on residue stability and the method efficiency for samples with incurred residues. The extract after partitioning contained anything from 2 to 40 mg/ml of oil residue for both extraction methods and depended on the variety and characteristics of the original oil, with a mean mass of residual oil of 11±7 mg/ml. Before further cleanup, the solvent from a 0.5 ml aliquot of the extract was evaporated under a gentle nitrogen stream and the residue was redissolved in 1 ml of acetonitrile. Alternatively, the residue from the extraction step was collected directly in acetonitrile.

2.5. Cleanup method development

Initial tests were made to assess the ability of different adsorbent materials to remove traces of oil remaining in extracts of olive oil after the initial extraction step. After prewashing each column with 3 ml acetonitrile, the oil extract dissolved in 1 ml acetonitrile was applied to the column and allowed to reach the surface of the adsorbent. The column was then eluted with 3 ml of acetonitrile. The eluate was injected directly into the gas chromatograph and the columns were assessed on the basis of interferences remaining in the ECD chromatogram after cleanup.

The four alumina-N columns were tested for recovery of the pesticides, using the method described above, with a mixture containing 1 μg/ml of each pesticide. In addition, the Isolute alumina-N cartridge and microcolumn with 1 g of alumina-N were tested with a second solvent system employing a prewash with 2 ml *n*-hexane, a load of 0.5 μg of each pesticide in 1 ml *n*-hexane, a column wash with 2 ml *n*-hexane and elution with 5 ml of a mixture of *n*-hexane–diethyl ether (7:3). The Sep-Pak column was further tested for the effects of deactivation of the alumina-N with two other solvents, acetonitrile with 1% water and methanol, for two different loads of pesticides in acetonitrile (1 and 0.1 μg). Recoveries were also measured for this cartridge from samples of olive oil fortified at 0.2 mg/kg after prewashes with (a) acetonitrile and (b) acetonitrile with 1% water added.

2.6. Method validation

The accuracy and precision of the method were assessed by the determination of five replicate recoveries at each of four fortification levels ranging from 0.05 (the estimated limit of quantification, LOQ, for most of the pyrethroids) to 1 mg/kg, and at the lower estimated LOQ of 0.02 mg/kg for α-endosulfan, β-endosulfan, endosulfan sulfate and λ-cyhalothrin. On replacement of the initial liquid–liquid partitioning method by the low-temperature precipitation method, recoveries were repeated as above for one fewer fortification level. The limit of detection (LOD) for each pesticide was calculated on the basis of three times the standard deviation for 10 repeat injections of a sample fortified at the estimated LOQ. The LOQ values were confirmed by the analysis of five replicate samples fortified at the estimated LOQ. Sensitivity and the linearity of the detector response to the analytes were examined by

the injection of matrix-matched standards at eight concentrations in the range 0.001 to 0.2 μg/ml.

2.7. Residue stability and comparative efficiency of the extraction methods for samples with incurred residues

Seven oil samples containing endosulfan residues, which had been stored in a freezer at −20°C for 2 years, were reanalysed with both extraction methods. The original extracts, which had been retained for GC–MS confirmation, were also reanalysed in order to assess the stability of the residues.

3. Results and discussion

3.1. Evaluation of cleanup columns

As anticipated on the basis of studies reported in the literature [10], the alumina-N columns showed a greater capacity for removing olive oil than the florisil and silica gel, and these were tested further. Recoveries for elution with acetonitrile from the Isolute alumina-N cartridge and the microcolumn with 500 mg of alumina-N were low (below 50%) and the former also gave the poorest cleaning effect when tested with oil extract. Increasing the amount of solid phase in the microcolumn to 1 g increased the recoveries for all the pesticides to above 60%, implying that the activity of the alumina may be too low. This was confirmed by the presence of pesticides in the fraction of acetonitrile eluting immediately after loading the sample onto the column. Recoveries for the Sep-Pak alumina-N column were all between 82 and 92%.

Recoveries for the *n*-hexane–diethyl ether (7:3) solvent system with the Isolute alumina-N cartridge and the microcolumn with 1 g of alumina-N were very variable, ranging from 60 to 209%, except for α-endosulfan on the Isolute cartridge, which showed a recovery below 15%.

Deactivation of the Sep-Pak alumina-N with (a) acetonitrile with 1% water and (b) methanol decreased the recoveries for some pesticides (1 and 0.1 μg loads of each pesticide in acetonitrile) compared to those obtained for deactivation with acetonitrile alone. However, the recoveries given in Table 1 for five replicate samples of olive oil fortified at 0.2 mg/kg after prewashes with (a) acetonitrile and (b) acetonitrile with 1% water show no great difference for the different solvent prewashes. Since a prewash with acetonitrile alone gave better cleanup with fewer interferences in the chromatogram, these results were not investigated further.

Table 1
Recoveries for samples fortified at 0.2 mg/kg after prewashes of Sep-Pak alumina-N cartridge with acetonitrile and acetonitrile with 1% water

Pesticide	Recovery (%)±RSD (%) ($n = 5$)	
	Acetonitrile	Acetonitrile with 1% water
α-Endosulfan	82±4	82±6
β-Endosulfan	91±2	95±3
Endosulfan sulfate	98±2	99±3
λ-Cyhalothrin	86±6	91±10
Permethrin	77±6	80±6
Cypermethrin	92±2	104±9
Fenvalerate	97±4	99±8
Deltamethrin	89±7	95±11

3.2. Final cleanup method

On the basis of the above tests, it was concluded that the Sep-Pak alumina-N cartridge with the acetonitrile solvent system provided a simple and satisfactory cleanup procedure. The following method was used for further validation work: a 500 mg Sep-Pak alumina-N cartridge was fitted with a 10 ml reservoir via a PTFE adapter and prewashed with 3 ml acetonitrile. The sample extract (corresponding to 0.5 g of oil) dissolved in 1 ml acetonitrile was loaded onto the cartridge. The sample container was rinsed with 1 ml of acetonitrile, which was then applied to the cartridge as soon as the solvent flow stopped. Collection of the eluate was begun at this point and elution was continued with a further 2 ml of acetonitrile when the solvent reached the bottom of the reservoir. The solvent usually passed through the cartridge under gravity up to the elution stage, when gentle pressure was applied to achieve a flow of approximately one drop per second. If necessary, pressure was applied at an earlier stage to obtain the required flow-rate. In this case, the first portion of eluting solvent was added when the solvent reached

the top of the closed cartridge and collection of the eluate was begun at this point. The final volume of extract was then 3.3 ml, rather than 3.1 ml when no pressure was applied at the prewash stage. The final volume was checked or adjusted to 3 ml exactly by evaporation with a gentle nitrogen stream.

3.3. Cleanup efficiency

For a typical sample of virgin olive oil with an amount of residual oil in the extract of 10 mg/ml of oil after the first extraction step, the oil remaining in the final extract after cleanup with a Sep-Pak alumina-N cartridge was 0.2 mg/ml, corresponding to a reduction of 88%. No difference was observed in either the amount of oil residue or the appearance of the chromatogram for the two different initial extraction steps. The cleanup achieved was sufficient for the chromatographic system to maintain its separation efficiency for at least 100 sample injections.

The appearance of oil peaks in the ECD chromatogram was found to be a function of the oil itself, with that from some varieties of olives giving rise to peaks which were absent in other samples. The intensity of the peaks also varied from sample to sample. Most samples were free of interferences on the HP-5MS, Rtx-50 and HP608 columns, while several small interferences on the Rtx-5 made this column less suitable for the analysis of samples with low concentrations of residues. The quantification of α-endosulfan on the Rtx-50 and HP608 columns was found to be unreliable because of a large interference peak of variable intensity. The HP-5MS column was, therefore, the most appropriate for the screening of samples for endosulfan, although in practice only a very small percentage of monitoring samples contained quantifiable concentrations of the parent compounds α- and β-endosulfan, and these were always much lower than the endosulfan sulfate concentration. Small interference peaks for *cis*-permethrin on the HP608 column and for one of the isomers of cypermethrin on the HP-5MS column prevented the reliable quantification of small concentrations of these compounds on these columns. Screening for their presence in samples was, however, possible in both cases because of the satisfactory detection of their isomers. Fig. 1 shows chromatograms of an oil sample extract and standards at a concentration of 0.02 μg/ml dissolved in the same extract on the HP-5MS and Rtx-50 columns.

3.4. Recoveries of pesticides

Recoveries for fortified samples extracted with the liquid–liquid partitioning step are given in Table 2. Those for samples for which the low-temperature precipitation method was used are given in Table 3. All recoveries lie within an acceptable range, although those for low-temperature precipitation are slightly lower for most compounds. This method is, however, preferred because of its simplicity and low cost. RSD values are between 6 and 17%, which is considered to be acceptable given the difficulty of analysing for these compound in virgin olive oil.

3.5. Limits of quantification, linearity and matrix effects

The LOQ values were evaluated several times over the 3-year period of the measurements and were found to be 0.02 mg/kg or better for endosulfan and λ-cyhalothrin and 0.05 mg/kg or better for the other pyrethroids for both GC systems and three of the four columns used, except where the interference peaks described above affected the analysis. The HP608 wide-bore capillary column gave LOQ values double the above. The ECD response was established to be linear ($R^2 > 0.99$) within the range 0.001–0.2 μg/ml (equivalent to 0.006–1.2 mg/kg) for endosulfan and λ-cyhalothrin and within the range 0.002–0.2 μg/ml (equivalent to 0.012–1.2 mg/kg) for the other pyrethroids. The matrix effect was found to be variable from system to system and over time, with the response to standards in solvent alone sometimes being greater and sometimes less than the response to matrix-matched standards. Matrix-matching of standards is considered to be necessary for the reliable quantification of all compounds [13–15].

3.6. Monitoring results

Residues of endosulfan, ranging from 0.02 to 0.57 mg/kg but mostly only slightly above the LOQ, were found in 22% of the 338 samples analysed. These were all at concentrations lower than the

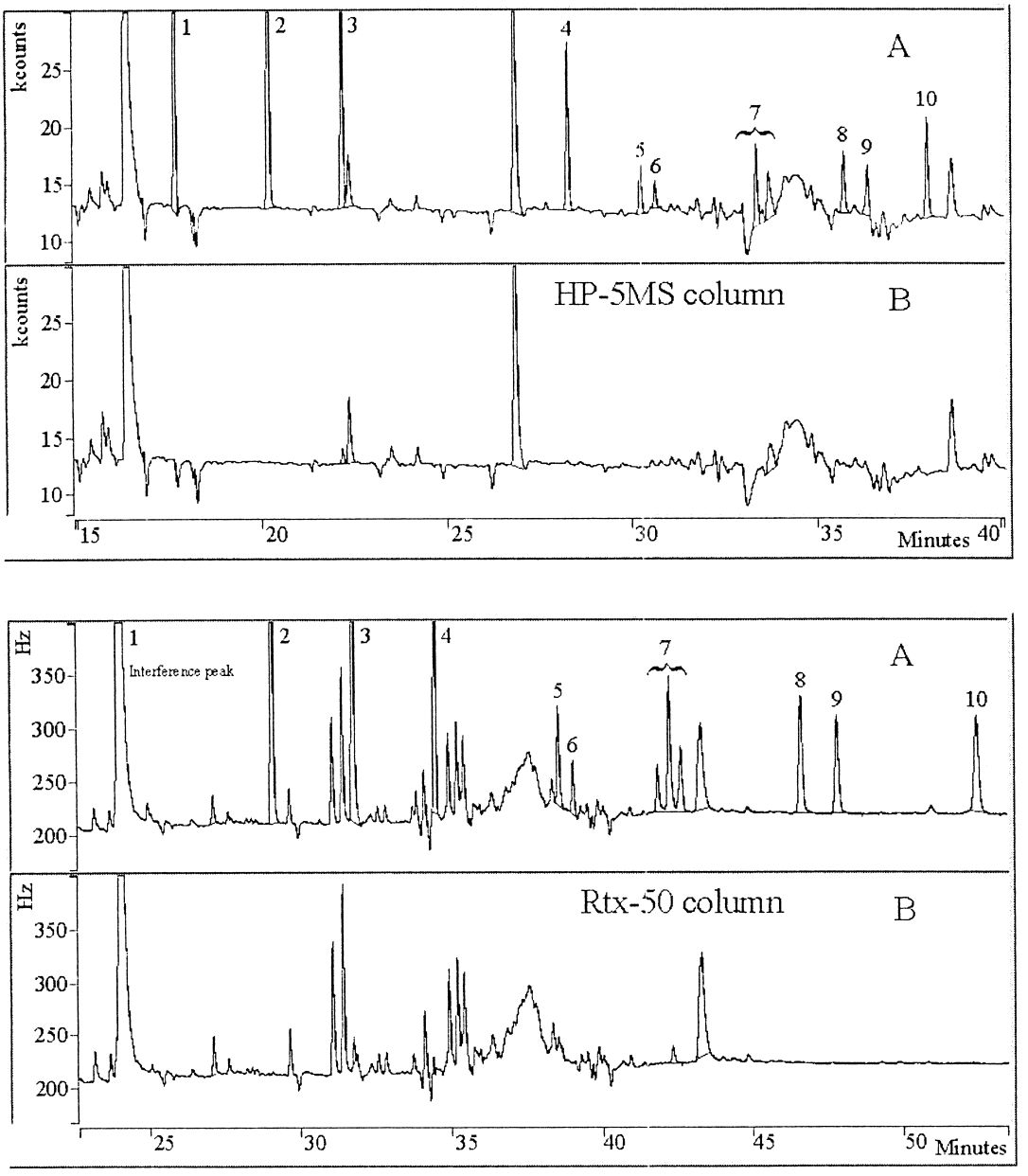

Fig. 1. Typical gas chromatograms of (A) 0.02 μg/ml standard mixture in oil extract and (B) the oil extract alone for HP-5MS and Rtx-50 columns (30 m×0.25 mm I.D., 0.25 μm film thickness). 1=α-Endosulfan, 2=β-endosulfan, 3=endosulfan sulfate, 4=λ-cyhalothrin, 5=*cis*-permethrin, 6=*trans*-permethrin, 7=cypermethrin, 8=fenvalerate I, 9=fenvalerate II, 10=deltamethrin.

European Union maximum residue levels (EU MRLs) for olives of 1 mg/kg, which refers to the sum of α-endosulfan, β-endosulfan and endosulfan sulfate [16]. The residues occurred almost exclusively as the sulfate metabolite, with only five samples also containing α- and β-endosulfan and two samples containing β-endosulfan above the LOQ. Two samples were found to contain residues of a pyre-

Table 2
Recoveries at different fortification levels using liquid–liquid partitioning followed by cleanup with Sep-Pak alumina-N cartridge

Pesticide	Recovery (%)±RSD (%) at given fortification level (mg/kg) ($n = 5$)					
	1	0.2	0.1	0.05	0.02	Overall
α-Endosulfan	–[a]	82±4	–[a]	66±5	72±8	73±12
β-Endosulfan	81±8	91±2	81±5	79±7	81±8	82±8
Endosulfan sulfate	87±9	98±2	94±4	85±8	91±10	91±8
λ-Cyhalothrin	91±11	86±6	107±4	74±11	82±9	88±17
Permethrin	68±13	77±6	85±13	67±8	–[b]	74±13
Cypermethrin	80±4	92±2	85±9	88±4	–[b]	86±8
Fenvalerate	89±12	97±4	90±7	79±9	–[b]	89±10
Deltamethrin	88±7	89±7	106±8	80±14	–[b]	91±16

[a] Not analysed because of interference peak on the HP608 column used.
[b] Fortification level below LOQ.

throid pesticide. These were λ-cyhalothrin at 0.02 mg/kg and cypermethrin at 0.04 mg/kg. Again, the concentrations determined were lower than the EU MRLs for olives of 0.02 mg/kg for λ-cyhalothrin [17] and 0.05 mg/kg for cypermethrin [18]. All residues were confirmed on a column of different polarity and endosulfan sulfate was confirmed using GC–MS.

3.7. Residue stability and comparison of extraction methods for samples with incurred residues

Data showing the stability of residues and the comparative efficiency of the two extraction methods for seven samples with incurred residues of endosulfan are shown in Table 4. No degradation of residues is apparent in either the oil extracts after cleanup or in the oil samples themselves after 2 years of storage at −20°C. There is good agreement between the results obtained using the two different extraction methods.

4. Conclusions

The method developed for the determination of residues of endosulfan and five pyrethroid insecticides in virgin olive oil is simple, inexpensive, efficient, and has the important advantage that it requires only small solvent volumes. It gives satisfactory recovery and repeatability for all the pesticides tested and good cleanup of the sample with very few interferences remaining in the later part of the ECD chromatogram where the pesticides elute. Its application to other oil matrices and screening for a wider range of pesticides should be possible.

Table 3
Recoveries at different fortification levels using low-temperature precipitation followed by cleanup with Sep-Pak alumina-N cartridge

Pesticide	Recovery (%)±RSD (%) at given fortification level (mg/kg) ($n = 5$)				
	1	0.2	0.05	0.02	Overall
α-Endosulfan	69±4	71±5	81±9	68±2[a]	72±9
β-Endosulfan	77±5	77±5	82±9	81±5[a]	79±7
Endosulfan sulfate	88±5	82±5	81±18	74±4[a]	82±11
λ-Cyhalothrin	79±6	83±17	78±14	101±12[a]	84±16
Permethrin	70±4	70±5	73±8	–[b]	71±6
Cypermethrin	80±5	79±8	84±2	–[b]	80±6
Fenvalerate	82±8	85±6	83±7	–[b]	83±7
Deltamethrin	73±5	84±13	78±18	–[b]	78±14

[a] $n = 4$.
[b] Fortification level below LOQ.

Table 4
Repeat analyses of samples with incurred residues of endosulfan using two different initial extraction methods

Sample	Form of endosulfan	Original determination (mg/kg)	Stored extract (mg/kg)	Repeat analysis after 2 years (mg/kg)	
				Liquid–liquid partitioning	Low-temperature precipitation
1	α	0.017	0.013	0.012	0.013
	β	0.022	0.020	0.020	0.017
	Sulfate	0.21	0.21	0.22	0.18
2	Sulfate	0.53	0.47	0.50	0.40
3	Sulfate	0.22	0.21	0.22	0.19
4	Sulfate	0.16	0.13	0.15	0.14
5	α	0.043	0.044	0.065	0.068
	β	0.033	0.030	0.032	0.035
	Sulfate	0.56	0.49	0.54	0.53
6	Sulfate	0.019	0.017	0.020	0.016
7	Sulfate	0.049	–[a]	0.039	0.040

[a] Not determined.

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